METHODS FOR TREATMENT AND PREVENTION OF GASTROINTESTINAL CONDITIONS

CROSS REFERENCE TO RELATED APPLICATIONS

Priority is claimed under Title 35 Untied States Code, §119 to United States Provisional application Serial No. 60/400,660, filed August 2, 2002, the disclosure of which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

The present invention relates in general to methods for treating gastrointestinal diseases and conditions, and more particularly to novel methods of treatment and prevention of conditions and diseases of the gastrointestinal tract, including ulceration, that involve an overproduction of nitric oxide.

Peptic ulcer disease is a chronic inflammatory condition of the stomach and duodenum that affects up to about ten percent of the United States population at some time during life. Although peptic ulcer disease does not have a high mortality rate, it nevertheless has a high economic cost, and results in serious distress for large numbers of individuals. Other forms of chronic inflammation in the upper gastrointestinal (G.I.) tract, such as superficial gastritis and esophagitis also result in substantial human suffering.

Until recently, treatment methods focused on the control of diet and stress-related factors in the belief that upper G.I. disease resulted primarily from the excessive secretion of digestive fluids such as gastric acid. Antacid therapy was the method of choice. In 1971, a subtype of the histamine receptor, the H_2 receptor, was first identified and believed to be the primary mediator of gastric acid secretion. H_2 receptor antagonists became available and were found to constitute safe and effective therapy for peptic ulcer disease. Later, other agents that enhance peptic mucosal defense, including proton pump inhibitors, bismuth compounds, sucralfate and prostaglandins

proved to be safe and effective agents for treatment. However, even with completely effective treatment, peptic ulcer disease has maintained a high rate of recurrence.

In 1982, the bacterium *Helicobacter pylori (H. pylori)* was first isolated from the narrow interface between the gastric epithelial cell surface and the overlying mucus gel layer. *H. pylori* was later identified as such and is also now known to be an important pathogen involved in gastroduodenal ulceration and carcinogenesis. While the pathology of *H. pylori* infection leading to inflammation and ulceration is not yet well understood, at least two possible mechanisms invoke the effect of *H. pylori* on levels of oxygen radicals. *H. pylori* may increase levels of oxygen radicals by inducing the release of oxygen radicals from neutrophils infiltrating inflamed gastric epithelium, or by inducing the production of oxygen radicals directly in the gastric epithelium. In either case, enhanced levels of oxygen radicals would enhance cell membrane damage.

While a causal relationship between *H. pylori* and peptic ulcer disease has not yet been established, the bacterium is clearly causally related to superficial gastritis. Almost all patients testing positive for *H. pylori* demonstrate antral gastritis, and elimination of *H. pylori* infection resolves gastritis. Chronic superficial gastritis is produced in animal models by intragastric administration of *H. pylori*, and at least two humans have been reported as developing gastritis upon oral administration of the bacterium. The most potent evidence for a causal link between *H. pylori* and peptic ulcer disease is a substantial decrease in recurrence rate upon eradication of *H. pylori* infection. Although the decrease is not so well established for gastric ulcers as for duodenal ulcers, the available evidence suggests a similar effect. Generally, the relationship between *H. pylori* infection and peptic ulcer disease has been more difficult to establish, perhaps because peptic ulcer disease lacks a suitable animal model, and because only a small fraction of infected

individuals actually develop ulceration.

Thus, in patients with gastritis, and patients with peptic ulcer disease who test positive for *H. pylori*, therapy now commonly includes administration of anti-microbials. However, the continuing lack of a suitable animal model for peptic ulcer disease has limited the ability to evaluate potential anti-microbial therapies. Data on the efficacy of anti-microbial therapy therefore largely depends on the limited trials that can be done in humans, and is currently evolving. Thus, no single standard of anti-microbial therapy exists in the case of peptic ulcer disease, and instead the choice of anti-microbial therapeutic regimens varies, necessarily taking into account a variety of factors including efficacy, compliance, side effects and cost. Agents that have been studied and employed include metronidazole, tetracycline, amoxicillin, clarithromycin, rifabutin, bismuth compounds, H_2 receptor antagonists, and proton-pump inhibitors, alone or in combination with one another.

Nitric oxide (NO) is now known to be a factor involved in inflammatory reactions in many body tissues. Nitric oxide is the factor responsible for the phenomenon of endothelium-dependent vascular relaxation that was first described in the 1980's. Since then, the biosynthesis of NO by the enzyme nitric oxide synthase (NOS) has been revealed, and we now know that NO is synthesized from the amino acid L-arginine by NOS. Nitric oxide is not, however, uniquely present in the vascular endothelium, but instead is generated in many different tissues in response to various stimuli, and appears to play varying physiological roles. In addition to endothelium-dependent vascular relaxation, NO is involved in numerous biological actions including, for example, cytotoxicity of phagocytic cells and cell-to-cell communication in the central nervous system. Nitric oxide is also an endogenous stimulator of the soluble guanylate cyclase. A growing body of evidence implicates NO in the degeneration of cartilage that takes place as a result of certain conditions

such as arthritis, and increased NO synthesis is associated with rheumatoid arthritis and osteoarthritis.

The precise role of NO in any given tissue under given conditions appears to be closely tied to the particular isoform of nitric oxide synthase that generates the NO. At least three types of NOS exist, as follows:

(i) a constitutive, Ca^{++}/calmodulin dependent enzyme, located in the endothelium (hereinafter "eNOS"), that releases NO in response to receptor or physical stimulation.

(ii) a constitutive, Ca^{++}/calmodulin dependent enzyme, located in the brain (hereinafter "nNOS"), that releases NO in response to receptor or physical stimulation.

(iii) a Ca^{++} independent enzyme which is induced after activation of vascular smooth muscle, macrophages, endothelial cells, and a number of other cells by endotoxin and cytokines. Once expressed this inducible nitric oxide synthase (hereinafter "iNOS") generates NO continuously for long periods.

The NO released by each of the two constitutive enzymes acts as a transduction mechanism underlying several physiological responses. In contrast, the NO produced by the inducible enzyme is a cytotoxic molecule for tumor cells, bacteria, viruses and parasites, and is thus a component of host defenses against cancers and invading microorganisms. However, it also appears that adverse effects of excess NO production, in particular pathological vasodilation and tissue damage, may result largely from the NO synthesized by iNOS. The large amounts of NO produced by iNOS are harmful to tissues by producing peroxynitrite resulting from the reaction of NO with superoxide. In the digestive system, increased iNOS activity associated with gastroduodenal inflammation may be linked to tissue damage leading to ulceration.

Increased iNOS activity may contribute to the tissue damage observed

with *H. pylori* infection of gastric epithelial cells. Increased iNOS activity is observed in patients with *H. pylori*-positive duodenal ulcers. Apoptosis, or programmed cell death, is induced by NO in several cell systems, and *H. pylori* infection results in apoptosis of gastric epithelial cells. Increased levels of iNOS expression and gastric epithelial cell apoptosis have been associated with *H. pylori* infection. Thus, chronically high levels of NO due to increased iNOS expression may be involved in H. pylori-induced gastric apoptosis.

Non-selective and selective inhibitors of NOS are known. More specifically, some of the NO synthase inhibitors proposed for therapeutic use are non-selective, in that they inhibit both the constitutive and the inducible NO synthases. Use of a non-selective NO synthase inhibitor therefore requires that great care be taken in order to avoid the potentially serious adverse effects of over-inhibition of the constitutive NO-synthase. Such adverse effects include hypertension and possible thrombosis and tissue damage. For example, in the case of the therapeutic use of the NOS inhibitor L-NMMA for the treatment of toxic shock it has been recommended that the patient must be subject to continuous blood pressure monitoring throughout the treatment. In particular, use of a non-selective NOS inhibitor that substantially interferes with the activity of eNOS may place a patient at risk of incurring damage to epithelial cells, including gastric epithelial cells, leading to possible gastric bleeding.

Thus, while methods of treatment and prevention of inflammatory conditions using non-selective NO synthase inhibitors might have therapeutic utility provided that appropriate precautions are taken, methods using NO synthase selective inhibitors, i.e. compounds that inhibit the inducible NO synthase to a considerably greater extent than the constitutive isoforms of NO synthase, would be of even greater therapeutic benefit and more easily practiced (S. Moncada and E. Higgs, FASEB J., 9, 1319-1330, 1995).

The following individual publications disclose compounds that inhibit nitric oxide synthesis and preferentially inhibit the inducible isoform of nitric oxide synthase:

PCT Patent Application No. WO 96/35677.

PCT Patent Application No. WO 96/33175.

PCT Patent Application No. WO 96/15120.

PCT Patent Application No. WO 95/11014.

PCT Patent Application No. WO 95/11231.

PCT Patent Application No. WO 99/46240.

PCT Patent Application No. WO 95/24382.

PCT Patent Application No. WO 94/12165.

PCT Patent Application No. WO 94/14780.

PCT Patent Application No. WO 93/13055.

PCT Patent Application No. WO 99/62875.

European Patent No. EP0446699A1.

U.S. Patent No. 5,132,453.

U.S. Patent No. 5,684,008.

U.S. Patent No. 5,830,917.

U.S. Patent No. 5,854,251.

U.S. Patent No. 5,863,931.

U.S. Patent No. 5,919,787.

U.S. Patent No. 5,945,408.

U.S. Patent No. 5,981,511.

U.S. Patent number 6,586,474 discloses certain amidino derivatives as being useful in inhibiting inducible nitric oxide synthase.

PCT Patent Application No. WO 99/62875 discloses further amidino compounds as being useful in inhibiting inducible nitric oxide synthase.

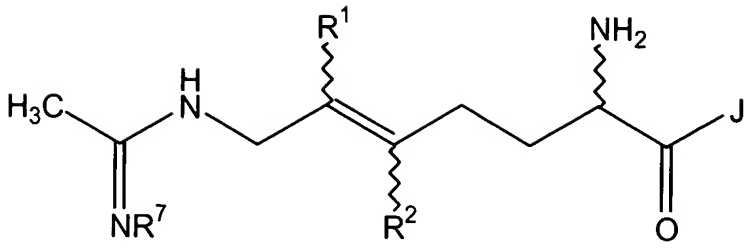
Against this background, increasing interest has developed in identifying new methods for treating conditions and diseases of the gastrointestinal tract

including but not limited to peptic ulcer disease and gastritis. Great interest also exists in identifying methods using combinations of low doses of two or more agents, each with different modes of action, so that overall treatment efficacy is improved while toxicity and adverse side effects of each agent are minimized. It would therefore be advantageous to identify and describe new methods for treating and preventing inflammatory conditions and diseases of the gastrointestinal tract that include the use of novel iNOS selective inhibitors. It would also be advantageous to identify and describe methods using combinations of iNOS selective inhibitors with other agents such as anti-microbials to maintain or improve the efficacy of each agent in the prevention and treatment of inflammatory conditions and diseases of the gastrointestinal tract.

SUMMARY OF THE INVENTION

Methods are described which will have the advantage of being efficacious in the treatment and prevention of conditions and diseases of the gastrointestinal tract that involve an overproduction of nitric oxide by iNOS, using novel compounds that act as iNOS selective inhibitors.

In a broad aspect, the present invention is directed to methods of using novel compounds and pharmaceutical compositions to treat or prevent conditions or diseases of the gastrointestinal tract that involve an overproduction of NO by iNOS, in a subject in need of such treatment or prevention, by administering to the subject an anti-inflammatory effective amount of an inducible nitric oxide synthase selective inhibitor or pharmaceutically acceptable salt thereof or prodrug thereof, wherein the inducible nitric oxide synthase inhibitor is selected from the group consisting of a compound having Formula I



I

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is selected from the group consisting of H, halo and alkyl which may be optionally substituted by one or more halo;

R^2 is selected from the group consisting of H, halo and alkyl which may be optionally substituted by one or more halo; with the proviso that at least one of R^1 or R^2 contains a halo;

R^7 is selected from the group consisting of H and hydroxy;

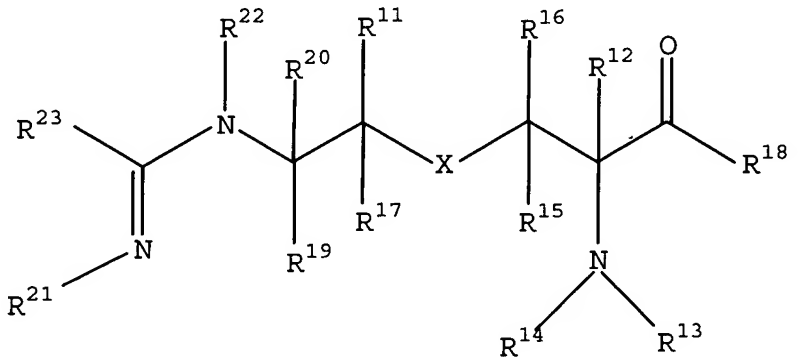
J is selected from the group consisting of hydroxy, alkoxy, and NR^3R^4 wherein;

R^3 is selected from the group consisting of H, lower alkyl, lower alkylenyl and lower alkynyl;

R^4 is selected from the group consisting of H, and a heterocyclic ring in which at least one member of the ring is carbon and in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur and said heterocyclic ring may be optionally substituted with heteroarylamino, N-aryl-N-alkylamino, N-heteroarylamino-N-alkylamino, haloalkylthio, alkanoyloxy, alkoxy, heteroaralkoxy, cycloalkoxy, cycloalkenyloxy, hydroxy, amino, thio, nitro, lower alkylamino, alkylthio, alkylthioalkyl, arylamino, aralkylamino, arylthio, alkylsulfinyl, alkylsulfonyl, alkylsulfonamido, alkylaminosulfonyl, amidosulfonyl, monoalkyl amidosulfonyl, dialkyl amidosulfonyl, monoarylamidosulfonyl, arylsulfonamido, diarylamidosulfonyl, monoalkyl monoaryl amidosulfonyl, arylsulfinyl, arylsulfonyl, heteroarylthio,

heteroarylsulfinyl, heteroarylsulfonyl, alkanoyl, alkenoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, haloalkanoyl, alkyl, alkenyl, alkynyl, alkylenedioxy, haloalkylenedioxy, cycloalkyl, cycloalkenyl, lower cycloalkylalkyl, lower cycloalkenylalkyl, halo, haloalkyl, haloalkoxy, hydroxyhaloalkyl, hydroxyaralkyl, hydroxyalkyl, hydoxyheteroaralkyl, haloalkoxyalkyl, aryl, aralkyl, aryloxy, aralkoxy, aryloxyalkyl, saturated heterocyclyl, partially saturated heterocyclyl, heteroaryl, heteroaryloxy, heteroaryloxyalkyl, arylalkyl, heteroarylalkyl, arylalkenyl, heteroarylalkenyl, cyanoalkyl, dicyanoalkyl, carboxamidoalkyl, dicarboxamidoalkyl, cyanocarboalkoxyalkyl, carboalkoxyalkyl, dicarboalkoxyalkyl, cyanocycloalkyl, dicyanocycloalkyl, carboxamidocycloalkyl, dicarboxamidocycloalkyl, carboalkoxycyanocycloalkyl, carboalkoxycycloalkyl, dicarboalkoxycycloalkyl, formylalkyl, acylalkyl, dialkoxyphosphonoalkyl, diaralkoxyphosphonoalkyl, phosphonoalkyl, dialkoxyphosphonoalkoxy, diaralkoxyphosphonoalkoxy, phosphonoalkoxy, dialkoxyphosphonoalkylamino, diaralkoxyphosphonoalkylamino, phosphonoalkylamino, dialkoxyphosphonoalkyl, diaralkoxyphosphonoalkyl, guanidino, amidino, and acylamino;

a compound having a structure corresponding to Formula II



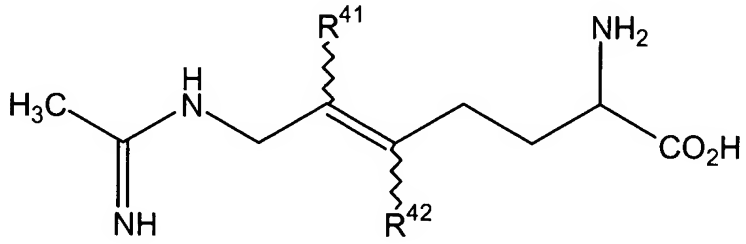
II

or a pharmaceutically acceptable salt thereof, wherein X is selected from the group consisting of -S-, -S(O)-, and -S(O)$_2$-. Preferably, X is -S-. R^{12}

is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_5 alkoxy-C_1 alkyl, and C_1-C_5 alkylthio-C_1 alkyl wherein each of these groups is optionally substituted by one or more substituents selected from the group consisting of -OH, alkoxy, and halogen. Preferably, R^{12} is C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH, alkoxy, and halogen. With respect to R^{13} and R^{18}, R^{18} is selected from the group consisting of $-OR^{24}$ and $-N(R^{25})(R^{26})$, and R^{13} is selected from the group consisting of -H, -OH, $-C(O)-R^{27}$, $-C(O)-O-R^{28}$, and $-C(O)-S-R^{29}$; or R^{18} is $-N(R^{30})-$, and R^{13} is -C(O)-, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring; or R^{18} is -O-, and R^{13} is $-C(R^{31})(R^{32})-$, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring. If R^{13} is $-C(R3^{21})(R^{32})-$, then R^{14} is $-C(O)-O-R^{33}$; otherwise R^{14} is -H. R^{11}, R^{15}, R^{16}, and R^{17} independently are selected from the group consisting of -H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_5 alkoxy-C_1 alkyl. R^{19} and R^{20} independently are selected from the group consisting of -H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_5 alkoxy-C_1 alkyl. With respect to R^{21} and R^{22}, R^{21} is selected from the group consisting of -H, -OH, $-C(O)-O-R^{34}$, and $-C(O)-S-R^{35}$, and R^{22} is selected from the group consisting of -H, -OH, $-C(O)-O-R^{36}$, and $-C(O)-S-R^{37}$; or R^{21} is -O-, and R^{22} is -C(O)-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring; or R^{21} is -C(O)-, and R^{22} is -O-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring. R^{23} is C_1 alkyl. R^{24} is selected from the group consisting of -H and C_1-C_6 alkyl, wherein when R^{24} is C_1-C_6 alkyl, R^{24} is optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. With respect to R^{25} and R^{26}, R^{25} is selected from the group consisting of -H, alkyl, and alkoxy, and R^{26} is selected from the group consisting of -H, -OH, alkyl, alkoxy, $-C(O)-R^{38}$, $-C(O)-O-R^{39}$, and $-C(O)-S-R^{40}$; wherein when R^{25} and R^{26} independently are alkyl or alkoxy, R^{25} and R^{26} independently are optionally substituted with one or more moieties selected

from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl; or R^{25} is -H; and R^{26} is selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35}, R^{36}, R^{37}, R^{38}, R^{39}, and R^{40} independently are selected from the group consisting of -H and alkyl, wherein alkyl is optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. When any of R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, $R19^{9}$, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35} R^{36}, R^{37}, R^{38}, R^{39}, and R^{40} independently is a moiety selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthio, cycloalkyl, heterocyclyl, aryl, and heteroaryl, then the moiety is optionally substituted by one or more substituent selected from the group consisting of -OH, alkoxy, and halogen;

a compound having Formula **III**



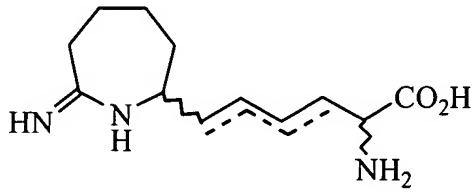
III

or a pharmaceutically acceptable salt thereof, wherein:

R^{41} is H or methyl; and

R^{42} is H or methyl;

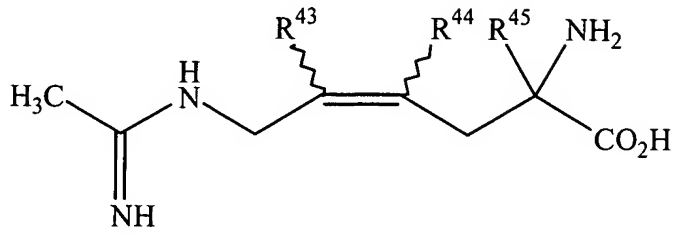
a compound having formula **IV**



IV

or a pharmaceutically acceptable salt thereof;

a compound having Formula **V**:



V

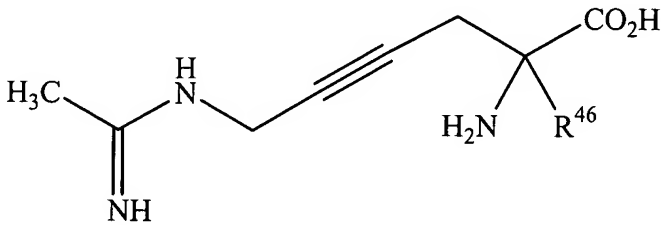
or a pharmaceutically acceptable salt thereof, wherein:

R^{43} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{44} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{45} is C_1-C_5 alkyl or C_1-C_5 alkyl be substituted by alkoxy or one or more halo;

a compound having Formula **VI**:

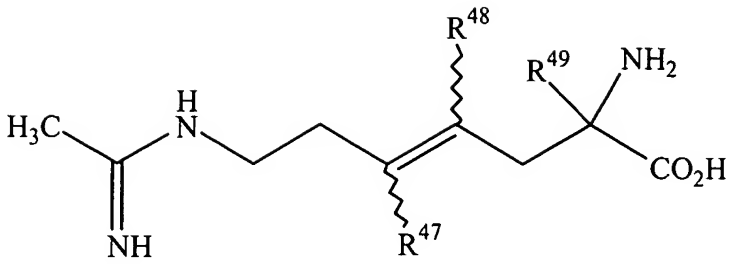


VI

or a pharmaceutically acceptable salt thereof, wherein:

R^{46} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

a compound having Formula **VII**



VII

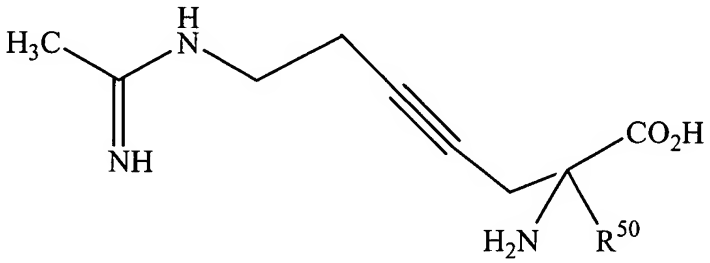
or a pharmaceutically acceptable salt thereof, wherein:

R^{47} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{48} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{49} is C_1-C_5 alkyl or C_1-C_5 alkyl be substituted by alkoxy or one or more halo;

a compound having Formula **VIII**

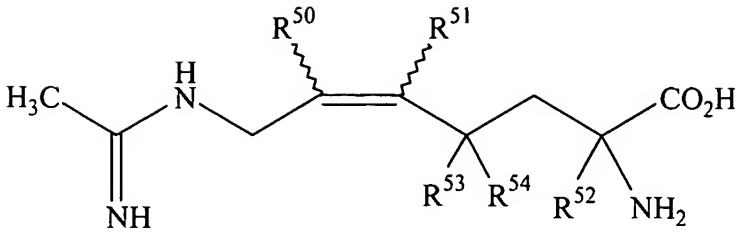


VIII

or a pharmaceutically acceptable salt thereof, wherein:

R^{50} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

a compound having Formula **IX**



IX

or a pharmaceutically acceptable salt thereof, wherein:

R^{50} is selected from the group consisting of hydrogen, halo, and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

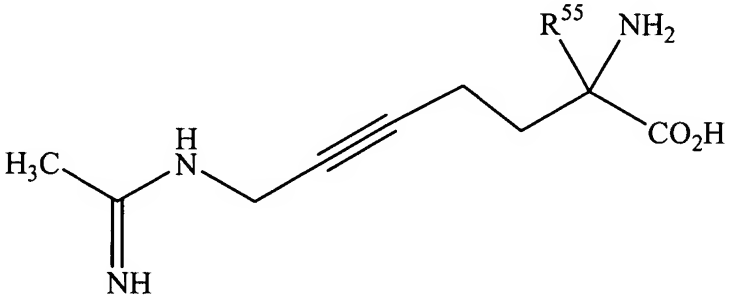
R^{51} is selected from the group consisting of hydrogen, halo, and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

R^{52} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

R^{53} is selected from the group consisting of hydrogen, halo, andC_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo; and

R^{54} is selected from the group consisting of halo and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

a compound having Formula **X**

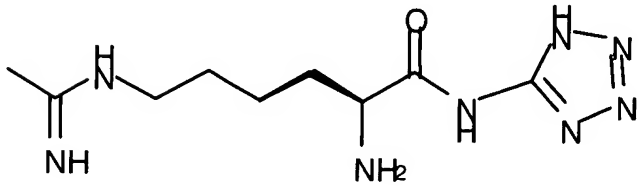


X

or a pharmaceutically acceptable salt thereof, wherein:

R^{55} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo; and

a compound of formula **XI**



2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazol-5-yl) hexanamide, hydrate, dihydrochloride

XI

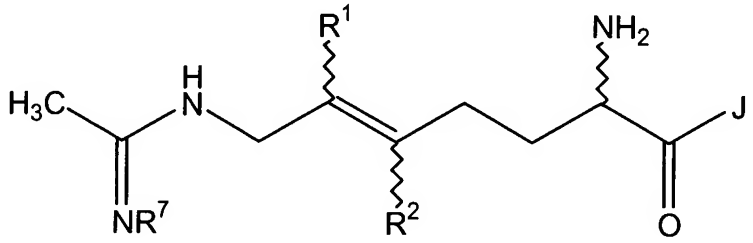
or a pharmaceutically acceptable salt thereof.

Conditions or diseases of the gastrointestinal tract that are treated or prevented using the methods of the present invention include, without limitation, inflammatory bowel disease including Crohn's disease and ulcerative colitis, peptic ulcer disease including gastric ulceration and duodenal ulceration, gastritis, colitis, ileitis, esophagitis, gastroesophageal reflux disease, irritable bowel syndrome, paralytic ileus and diarrhea.

The methods of the present invention also include methods for the treatment or prevention of conditions or diseases of the gastrointestinal tract involving an overproduction of nitric oxide (NO) by inducible nitric oxide synthase (iNOS) and microbial infection, in a subject in need of such treatment

or prevention, wherein the method includes administering to the subject an amount of an inducible nitric oxide synthase selective inhibitor or pharmaceutically acceptable salt thereof or prodrug thereof, and an amount of an antimicrobial compound or pharmaceutically acceptable salt thereof or prodrug thereof, wherein the amount of the inducible nitric oxide synthase selective inhibitor and the amount of the antibiotic compound together constitute an amount effective against conditions and diseases of the gastrointestinal tract, and the inducible nitric oxide synthase inhibitor is selected from the group consisting of:

a compound having Formula I



I

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is selected from the group consisting of H, halo and alkyl which may be optionally substituted by one or more halo;

R^2 is selected from the group consisting of H, halo and alkyl which may be optionally substituted by one or more halo;

with the proviso that at least one of R^1 or R^2 contains a halo;

R^7 is selected from the group consisting of H and hydroxy;

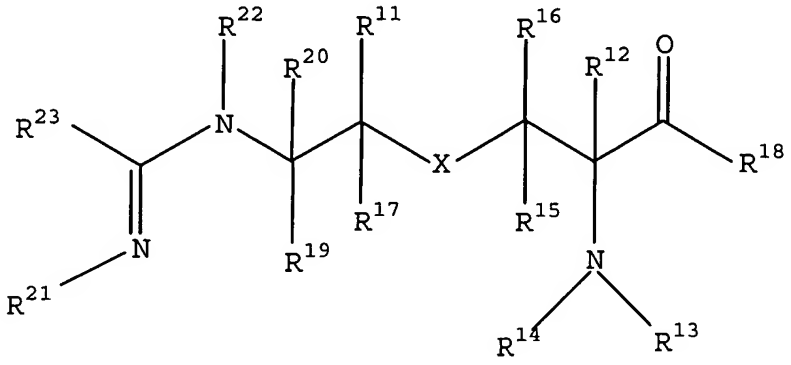
J is selected from the group consisting of hydroxy, alkoxy, and NR^3R^4 wherein;

R^3 is selected from the group consisting of H, lower alkyl, lower alkylenyl and lower alkynyl;

R^4 is selected from the group consisting of H, and a heterocyclic ring in which at least one member of the ring is carbon and in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur and said heterocyclic ring may be optionally substituted with heteroarylamino, N-aryl-N-alkylamino, N-heteroarylamino-N-alkylamino, haloalkylthio, alkanoyloxy, alkoxy, heteroaralkoxy, cycloalkoxy, cycloalkenyloxy, hydroxy, amino, thio, nitro, lower alkylamino, alkylthio, alkylthioalkyl, arylamino, aralkylamino, arylthio, alkylsulfinyl, alkylsulfonyl, alkylsulfonamido, alkylaminosulfonyl, amidosulfonyl, monoalkyl amidosulfonyl, dialkyl amidosulfonyl, monoarylamidosulfonyl, arylsulfonamido, diarylamidosulfonyl, monoalkyl monoaryl amidosulfonyl, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, alkanoyl, alkenoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, haloalkanoyl, alkyl, alkenyl, alkynyl, alkylenedioxy, haloalkylenedioxy, cycloalkyl, cycloalkenyl, lower cycloalkylalkyl, lower cycloalkenylalkyl, halo, haloalkyl, haloalkoxy, hydroxyhaloalkyl, hydroxyaralkyl, hydroxyalkyl, hydoxyheteroaralkyl, haloalkoxyalkyl, aryl, aralkyl, aryloxy, aralkoxy, aryloxyalkyl, saturated heterocyclyl, partially saturated heterocyclyl, heteroaryl, heteroaryloxy, heteroaryloxyalkyl, arylalkyl, heteroarylalkyl, arylalkenyl, heteroarylalkenyl, cyanoalkyl, dicyanoalkyl, carboxamidoalkyl, dicarboxamidoalkyl, cyanocarboalkoxyalkyl, carboalkoxyalkyl, dicarboalkoxyalkyl, cyanocycloalkyl, dicyanocycloalkyl, carboxamidocycloalkyl, dicarboxamidocycloalkyl, carboalkoxycyanocycloalkyl, carboalkoxycycloalkyl, dicarboalkoxycycloalkyl, formylalkyl, acylalkyl, dialkoxyphosphonoalkyl, diaralkoxyphosphonoalkyl, phosphonoalkyl, dialkoxyphosphonoalkoxy, diaralkoxyphosphonoalkoxy, phosphonoalkoxy, dialkoxyphosphonoalkylamino, diaralkoxyphosphonoalkylamino,

phosphonoalkylamino, dialkoxyphosphonoalkyl, diaralkoxyphosphonoalkyl, guanidino, amidino, and acylamino;

a compound having a structure corresponding to Formula II

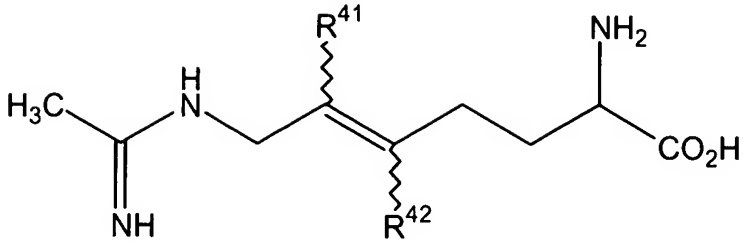


II

or a pharmaceutically acceptable salt thereof, wherein X is selected from the group consisting of -S-, -S(O)-, and -$S(O)_2$-. Preferably, X is -S-. R^{12} is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_5 alkoxy-C_1 alkyl, and C_1-C_5 alkylthio-C_1 alkyl wherein each of these groups is optionally substituted by one or more substituent selected from the group consisting of -OH, alkoxy, and halogen. Preferably, R^{12} is C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH, alkoxy, and halogen. With respect to R^{13} and R^{18}, R^{18} is selected from the group consisting of $-OR^{24}$ and -$N(R^{25})(R^{26})$, and R^{13} is selected from the group consisting of -H, -OH, -C(O)-R^{27}, -C(O)-O-R^{28}, and -C(O)-S-R^{29}; or R^{18} is -$N(R^{30})$-, and R^{13} is -C(O)-, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring; or R^{18} is -O-, and R^{13} is -$C(R^{31})(R^{32})$-, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring. If R^{13} is -$C(R3^{21})(R^{32})$-, then R^{14} is -C(O)-O-R^{33}; otherwise R^{14} is -H. R^{11}, R^{15}, R^{16}, and R^{17} independently are selected from the group consisting of -H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_5 alkoxy-C_1 alkyl. R^{19} and R^{20} independently are selected from the group consisting of -H, C_1-C_6 alkyl, C_2-C_6

alkenyl, C_2-C_6 alkynyl, and C_1-C_5 alkoxy-C_1 alkyl. With respect to R^{21} and R^{22}, R^{21} is selected from the group consisting of -H, -OH, -C(O)-O-R^{34}, and -C(O)-S-R^{35}, and R^{22} is selected from the group consisting of -H, -OH, -C(O)-O-R^{36}, and -C(O)-S-R^{37}; or R^{21} is -O-, and R^{22} is -C(O)-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring; or R^{21} is -C(O)-, and R^{22} is -O-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring. R^{23} is C_1 alkyl. R^{24} is selected from the group consisting of -H and C_1-C_6 alkyl, wherein when R^{24} is C_1-C_6 alkyl, R^{24} is optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. With respect to R^{25} and R^{26}, R^{25} is selected from the group consisting of -H, alkyl, and alkoxy, and R^{26} is selected from the group consisting of -H, -OH, alkyl, alkoxy, -C(O)-R^{38}, -C(O)-O-R^{39}, and -C(O)-S-R^{40}; wherein when R^{25} and R^{26} independently are alkyl or alkoxy, R^{25} and R^{26} independently are optionally substituted with one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl; or R^{25} is -H; and R^{26} is selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35}, R^{36}, R^{37}, R^{38}, R^{39}, and R^{40} independently are selected from the group consisting of -H and alkyl, wherein alkyl is optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. When any of R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, $R19^{9}$, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35} R^{36}, R^{37}, R^{38}, R^{39}, and R^{40} independently is a moiety selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthio, cycloalkyl, heterocyclyl, aryl, and heteroaryl, then the moiety is optionally substituted by one or more substituent selected from the group consisting of -OH, alkoxy, and halogen;

a compound represented by Formula **III**



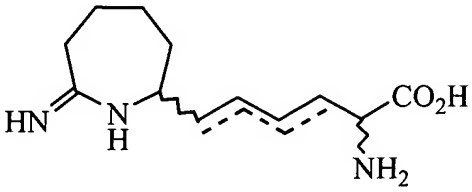
III

or a pharmaceutically acceptable salt thereof, wherein:

R^{41} is H or methyl; and

R^{42} is H or methyl;

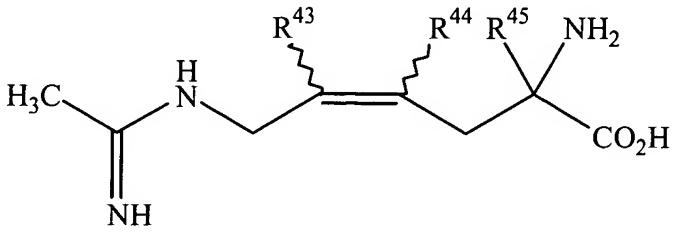
a compound of formula **IV**



IV

or a pharmaceutically acceptable salt thereof;

a compound of Formula **V**:



V

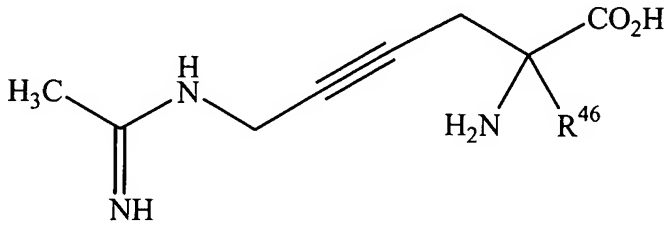
or a pharmaceutically acceptable salt thereof, wherein:

R^{43} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{44} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{45} is C_1-C_5 alkyl or C_1-C_5 alkyl be substituted by alkoxy or one or more halo;

a compound of Formula **VI**:

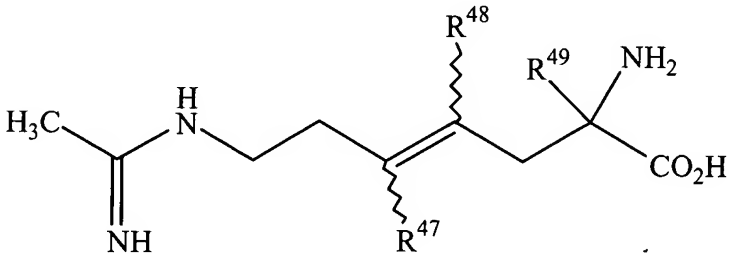


VI

or a pharmaceutically acceptable salt thereof, wherein:

R^{46} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

a compound of Formula **VII**



VII

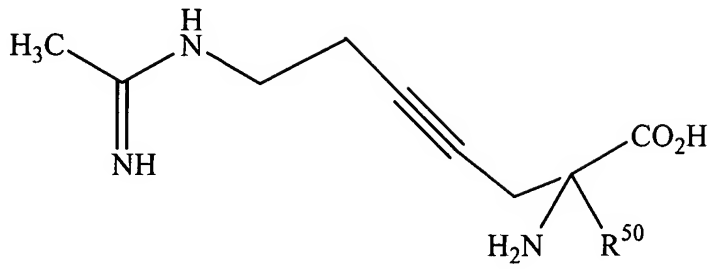
or a pharmaceutically acceptable salt thereof, wherein:

R^{47} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{48} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{49} is C_1-C_5 alkyl or C_1-C_5 alkyl be substituted by alkoxy or one or more halo;

a compound of Formula **VIII**

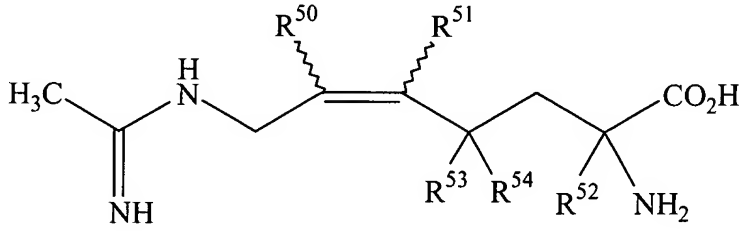


VIII

or a pharmaceutically acceptable salt thereof, wherein:

R^{50} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

a compound of formula **IX**



IX

or a pharmaceutically acceptable salt thereof, wherein:

R^{50} is selected from the group consisting of hydrogen, halo, and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

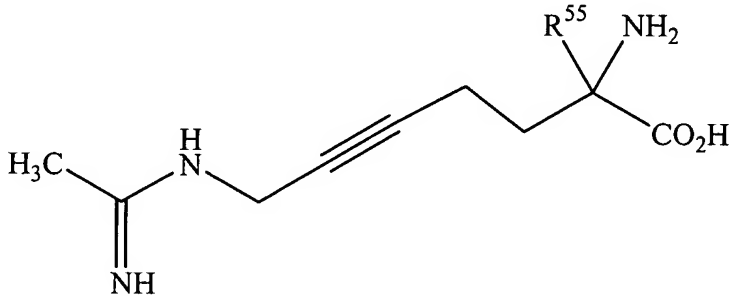
R^{51} is selected from the group consisting of hydrogen, halo, and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

R^{52} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

R^{53} is selected from the group consisting of hydrogen, halo, andC_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo; and

R^{54} is selected from the group consisting of halo and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo; and

a compound of formula **X**

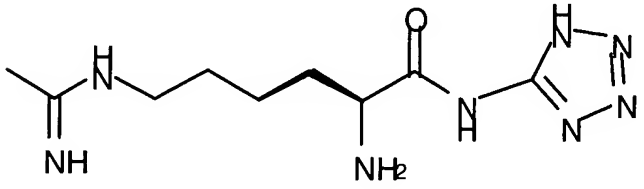


X

or a pharmaceutically acceptable salt thereof, wherein:

R^{55} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo.

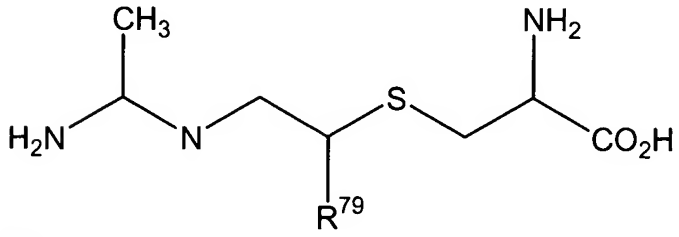
In another exemplary compound, the inducible nitric oxide synthase selective inhibitor is the compound having the formula XI, or a pharmaceutically acceptable thereof. Compound XI has previously been described in International Publication Number WO 00/26195, published May 11, 2000, which is herein incorporated by reference.



XI

2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazol-5-yl) hexanamide, hydrate, dihydrochloride

The invention also contemplates use of other selective iNOS inhibitors. By way of example, iNOS selective inhibitors also useful in the present invention are described in U.S. Patent No. 6,355,689, Beswick et al., filed November 29, 2000 and issued March 12, 2002, which describes and claims a selective iNOS inhibitor with the formula XII:



XII

wherein R^{79} is selected from C_{1-4} alkyl, C_{3-4} cycloalkyl, C_{1-4} hydroxyalkyl, and C_{1-4} haloalkyl. The description of U.S. Patent 6,355,689 states that R^{79} is preferably C_{1-4} alkyl, and most preferably, methyl. Specific embodiments disclosed in US Patent 6,355,689 and suitable for use in the present methods and compositions include:

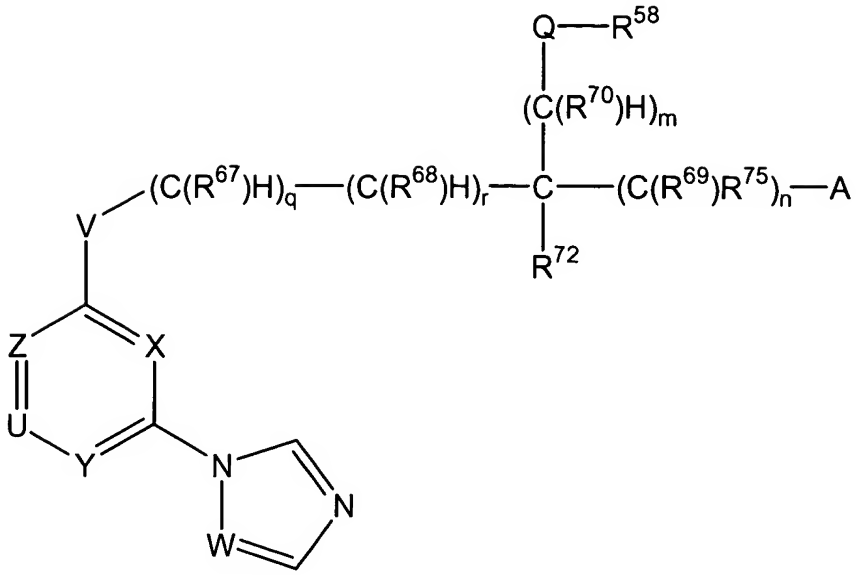
S-((R)-2-(1-iminoethylamino)propyl)-L-cysteine;
S-((S)-2-(1-iminoethylamino)propyl)-L-cysteine;
S-((R/S)-2-(1-iminoethylamino)propyl)-L-cysteine;
S-((R)-2-(1-iminoethylamino)propyl)-D-cysteine;
S-((S)-2-(1-iminoethylamino)propyl)-D-cysteine;
S-((R/S)-2-(1-iminoethylamino)propyl)-D-cysteine;
S-((R/S)-2-(1-iminoethylamino)butyl)-L-cysteine;
S-((R/S)-2-(1-iminoethylamino,2-cyclopropyl)ethyl)-L-cysteine; and

S-((R/S)-2-(1-iminoethylamino,3-hydroxy)propyl)-L-cysteine,

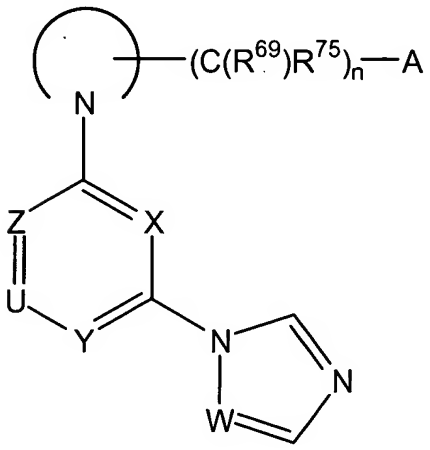
or a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof.

The above selective iNOS inhibitors are believed to work by competing with arginine as a substrate for the iNOS enzyme. Another strategy for inhibition of iNOS has been described by Arnaiz et al. in international patent application number PCT/US98/03176, publication number WO 98/37079 (Berlex Laboratories, Inc. Richmond, CA 94804-0099 and Pharmacopeia, Inc. Princeton, NJ 08540), published August 27, 1998 (Arnaiz). The Arnaiz application describes inhibitors of iNOS monomer dimerization. The iNOS enzyme is a homodimer; each monomer has a reductase domain, incorporating binding sites for flavin cofactors (FAD and FMN) and for NADPH. The reductase domain supplies electrons to the oxidase domain of the other monomer, where L-arginine is oxidized at the active site, which incorporates a heme group (Fe) cytochrome P-450 domain. Tetrahydrobiopterin (BH4) is required for homodimerization and modulates the heme redox state during electron transfer. iNOS monomers are inactive, and dimerization is required for activity.

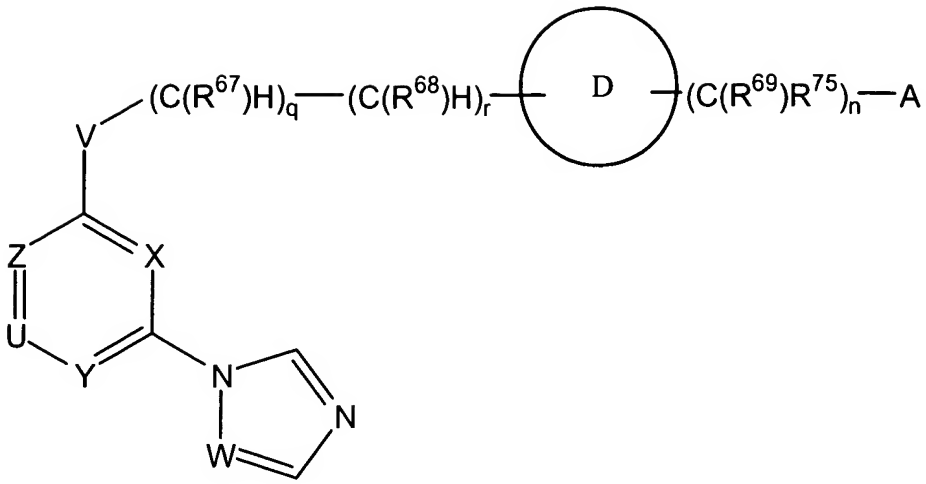
Thus, in another embodiment of the present invention, the selective iNOS inhibitor is a dimerization inhibitor represented by a compound of Formula XIII, Formula XIV or Formula XV:



Formula XIII;



Formula XIV; or



Formula XV;

wherein:

A is $-R^{56}$, $-OR^{56}$, $C(O)N(R^{56})R^{57}$, $P(O)[N(R^{56})R^{57}]_2$, $-N(R^{56})C(O)R^{57}$, $-N(R^{76})C(O)OR^{56}$, $-N(R^{56})R^{76}$, $-N(R^{71})C(O)N(R^{56})R^{71}$, $-S(O)_tR^{56}$, $-SO_2NHC(O)R^{56}$, $-NHSO_2R^{77}$, $-SO_2NH(R^{56})H$, $-C(O)NHSO_2R^{77}$, and $-CH=NOR^{56}$;

each X, Y and Z are independently N or $C(R^{19})$;

each U is N or $C(R^{60})$, provided that U is N only when X is N and Z and Y are CR^{74};

V is $N(R^{59})$, S, O or $C(R^{59})H$;

Each W is N or CH;

Q is chosen from the group consisting of a direct bond, -C(O)-, -O-, $-C(=N-R^{56})-$, $S(O)_t$, and $-N(R^{61})-$;

m is zero or an integer from 1 to 4;

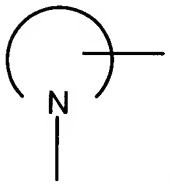
n is zero or an integer from 1 to 3;

q is zero or one;

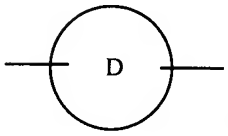
r is zero or one, provided that when Q and V are heteroatoms, m, q, and r cannot all be zero;

when A is $-OR^{56}$, $N(R^{56})C(O)R^{57}$, $-N(R^{71})C(O)OR^{57}$, $-N(R^{56})R^{76}$, $-N(R^{71})C(O)N(R^{56})R^{71}$, $-S(O)_tR^{56}$ (where t is zero), or $-NHSO_2R^{77}$, n, q, and r cannot all be zero; and when Q is a heteroatom and A is $-OR^{56}$, $N(R^{56})C(O)R^{57}$, $-N(R^{71})C(O)OR^{57}$, $-N(R^{56})R^{76}$, $N(R^{71})C(O)N(R^{56})R^{71}$, $-S(O)_tR^{56}$ (when t is zero), or $-NHSO_2R^{77}$, m and n cannot both be zero;

t is zero, one or two;



is an optionally substituted N-heterocyclyl;



is an optionally substituted carbocyclyl or optionally substituted N-heterocyclyl;

each R^{56} and R^{57} are independently chosen from the group consisting of hydrogen, optionally substituted C_1-C_{20} alkyl, optionally substituted cycloalkyl, -[C_0-C_8 alkyl]-R^{64}, -[C_2-C_8 alkenyl]-R^{64}, -[C_2-C_8 alkynyl]-R^{64}, -[C_2-C_8 alkyl]-R^{65} (optionally substituted by hydroxy), -[C_1-C_8]-R^{66} (optionally substituted by hydroxy), optionally substituted heterocyclyl;

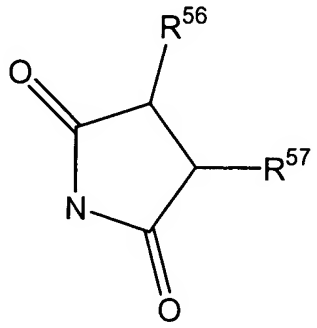
or R^{56} and R^{57} together with the nitrogen atom to which they are attached is an optionally substituted N-heterocyclyl;

R^{58} is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, optionally substituted aryl, haloalkyl, -[C_1-C_8 alkyl]-$C(O)N(R^{56})R^{57}$, -[C_1-C_8 alkyl]- $N(R^{56})R^{57}$, -[C_1-C_8 alkyl]-R^{63}, -[C_2-C_8 alk2yl]-R^{65}, -[C_1-C_8 alkyl]-R^{66}, and heterocyclyl (optionally substituted by one or more substitutents selected from the group consisting of halo, alkyl, alkoxy and imidazolyl);

or when Q is $-N(R^{58})-$ or a direct bond to R^{58}, R^{58} may additionally be aminocarbonyl,

alkoxycarbonyl, alkylsulfonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl and $-C(=NR^{73})-NH_2$;

or $-Q-R^{58}$ taken together represents $-C(O)OH$, $-C(O)N(R^{56})R^{57}$ or



;

R^{59} is chosen from the group consisting of hydrogen, alkyl, aryl, aralkyl and cycloalkyl;

Provided that when A is $-R^{56}$ or $-OR^{56}$, R^{59} cannot be hydrogen, and when V is CH, R^{59} may additionally be hydroxy;

R^{60} is chosen from the group consisting of hydrogen, alkyl, aryl, aralkyl, haloalkyl,

optionally substituted aralkyl, optionally substituted aryl, $-OR^{71}$, $-S(O)_t-R^{71}$, $N(R^{71})R^{76}$, $N(R^{71})C(O)N(R^{56})R^{71}$, $N(R^{71})C(O)OR^{71}$, $N(R^{71})C(O)\ R^{71}$, $-[C_0-C_8\ alkyl]-C(H)[C(O)R^{71}]_2$ and $-[C_0-C_8\ alkyl]-\ C(O)N(R^{56})R^{71}$;

R^{61} is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, $-[C_1-C_8\ alkyl]-R^{63}$, $-[C_2-C_8]alkyl]-R^{65}$, $-[C_1-C_8\ alkyl]-R^{66}$, acyl, $-C(O)R^{63}$, $-C(O)-\ -[C_1-C_8\ alkyl]-R^{63}$, alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted aralkoxycarbonyl, alkylsulfonyl, optionally substituted aryl, optionally substituted heterocyclyl, alkoxycarbonylalkyl, carboxyalkyl, optionally substituted arylsulfonyl, aminocarbonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl, optionally substituted arylaminocarbonyl, aminosulfonyl,

monoalkylaminosulfonyl dialkylaminosulfonyl, arylaminosulfonyl, arylsulfonylaminocarbonyl, optionally substituted N-heterocyclyl, -C(=NH)-N(CN)R^{56}, -C(O)R^{78}-N(R^{56})R^{57}, -C(O)-N(R^{56})R^{78}-C(O)OR^{56};

each R^{63} and R^{64} are independently chosen from the group consisting of haloalkyl,

cycloalkyl, (optionally substituted with halo, cyano, alkyl or alkoxy), carbocyclyl (optionally substituted with one or more substituents selected from the group consisting of halo, alkyl and alkoxy) and heterocyclyl (optionally substituted with alkyl, aralkyl or alkoxy);

each R^{65} is independently chosen from the group consisting of halo, alkoxy, optionally

substituted aryloxy, optionally substituted aralkoxy, optionally substituted –$S(O)_t$-R^{77}, acylamino, amino, monoalkylamino, dialkylamino, (triphenylmethyl)amino, hydroxy, mercapto, alkylsulfonamido;

each R^{66} is independently chosen from the group consisting of cyano, di(alkoxy)alkyl,

carboxy, alkoxycarbonyl, aminocarbonyl, monoalkylaminocarbonyl and dialkylaminocarbonyl;

each R^{67}, R^{68}, R^{69}, R^{70}, R^{72}, and R^{75} are independently hydrogen or alkyl;

each R^{71} is independently hydrogen, alkyl, optionally substituted aryl, optionally substituted aralkyl or cycloalkyl;

R^{73} is hydrogen, NO_2, or toluenesulfonyl;

each R^{74} is independently hydrogen, alkyl (optionally substituted with hydroxy), cyclopropyl, halo or haloalkyl;

each R^{76} is independently hydrogen, alkyl, cycloalkyl, optionally substituted aryl,

optionally substituted aralkyl, -C(O)R^{77} or –SO_2R^{77};

or R^{76} taken together with R^{56} and the nitrogen to which they are attached is an optionally

substituted N-heterocyclyl;

or R^{76} taken together with R^{71} and the nitrogen to which they are attached is an optionally

substituted N-heterocyclyl;

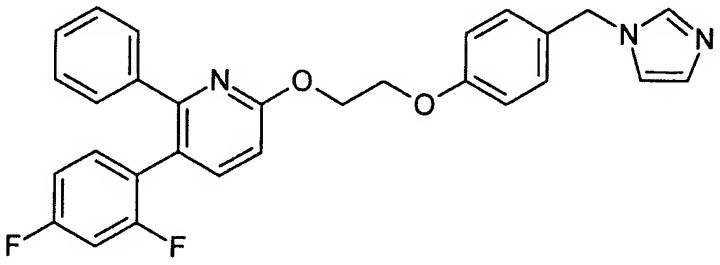
each R^{77} is independently alkyl, cycloalkyl, optionally substituted aryl or optionally

substituted aralkyl; and

R^{78} is an amino acid residue;

as a single stereoisomer or mixture thereof, or a pharmaceutically acceptable salt thereof.

Another iNOS dimerization inhibitor, 3-(2,4-difluorophenyl)-6-{2-[4-(1*H*-imidazol-1-ylmethyl) phenoxy]ethoxy}-2-phenylpyridine (PPA250) has been described in Ohtsuka et al., *J Phamacol Exp Ther* Vol. 303, Issue 1, 52-57, October 2002. PPA250 has the structure:



PPA250

Therefore, in another embodiment of the present invention, the compound PPA250 may be employed as the selective iNOS inhibitor.

The antimicrobial compound is, for example, a nitroimidazole, a proton-pump inhibitor, a bismuth compound, or any antibiotic compound such as penicillin. Antimicrobial compounds useful in combination with a selective iNOS inhibitor according to the methods of the present invention include amoxicillin, clarithromycin, rifabutin, bismuth subsalicylate, metronidazole, omeprarazole, ranitidine, and tetracycline, alone or in combination with one another. A double anti-microbial compound useful in the methods of the

present invention is, for example, a combination of omeprazole and amoxicillin. A triple anti-microbial compound useful in the methods of the present invention is, for example, a combination of ranitidine, metronidazole, and amoxicillin.

DETAILED DESCRIPTION OF THE INVENTION

The following detailed description is provided to aid those skilled in the art to practice the present invention. However, this detailed description should not be construed to unduly limit the present invention, inasmuch as modifications and variations in the exemplary embodiments discussed herein can be made by those of ordinary skill in the art without departing from the scope of the appended claims.

The contents of each of the primary references cited herein, including the contents of the references cited within the primary references, are herein incorporated by reference in their entirety.

The present invention encompasses therapeutic methods using novel selective iNOS inhibitors to treat or prevent inflammatory conditions or diseases of the gastrointestinal tract, including therapeutic methods of use in medicine for preventing and treating inflammatory bowel disease including Crohn's disease and ulcerative colitis, peptic ulcer disease including gastric ulceration, duodenal ulceration and esophageal ulceration, and other inflammatory conditions including gastritis, ileitis, esophagitis, gastroesophageal reflux disease, irritable bowel syndrome, paralytic ileus and diarrhea. The therapeutic methods include administering to a subject in need thereof an anti-inflammatory effective amount effective amount of a selective inhibitor of inducible nitric oxide synthase having a formula selected from Formulas I-X.

a. Definitions

The following definitions are provided in order to aid an understanding of the detailed description of the present invention:

The term "alkyl", alone or in combination, means an acyclic alkyl radical, linear or branched, preferably containing from 1 to about 10 carbon atoms and more preferably containing from 1 to about 6 carbon atoms. "Alkyl" also encompasses cyclic alkyl radicals containing from 3 to about 7 carbon atoms, preferably from 3 to 5 carbon atoms. Said alkyl radicals can be optionally substituted with groups as defined below. Examples of such radicals include methyl, ethyl, chloroethyl, hydroxyethyl, n-propyl, isopropyl, n-butyl, cyanobutyl, isobutyl, sec-butyl, tert-butyl, pentyl, aminopentyl, iso-amyl, hexyl, octyl and the like.

The term "alkenyl" refers to an unsaturated, acyclic hydrocarbon radical, linear or branched, in so much as it contains at least one double bond. Such radicals containing from 2 to about 6 carbon atoms, preferably from 2 to about 4 carbon atoms, more preferably from 2 to about 3 carbon atoms. Said alkenyl radicals may be optionally substituted with groups as defined below. Examples of suitable alkenyl radicals include propenyl, 2-chloropropylenyl, buten-1-yl, isobutenyl, penten-l-yl, 2-methylbuten-1-yl, 3-methylbuten-1-yl, hexen-1-yl, 3-hydroxyhexen-1-yl, hepten-1-yl, and octen-1-yl, and the like.

The term "alkynyl" refers to an unsaturated, acyclic hydrocarbon radical, linear or branched, in so much as it contains one or more triple bonds, such radicals containing 2 to about 6 carbon atoms, preferably from 2 to about 4 carbon atoms, more preferably from 2 to about 3 carbon atoms. Said alkynyl radicals may be optionally substituted with groups as defined below. Examples of suitable alkynyl radicals include ethynyl, propynyl, hydroxypropynyl, butyn-1-yl, butyn-2-yl, pentyn-1-yl, pentyn-2-yl, 4-methoxypentyn-2-yl, 3-methylbutyn-1-yl, hexyn-1-yl, hexyn-2-yl, hexyn-3-yl, 3,3-dimethylbutyn-1-yl radicals and the like.

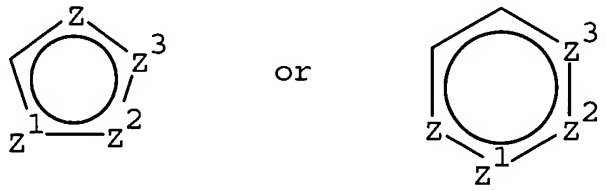
The term "alkoxy" embrace linear or branched oxy-containing radicals each having alkyl portions of 1 to about 6 carbon atoms, preferably 1 to about 3 carbon atoms, such as a methoxy radical. The term "alkoxyalkyl" also embraces alkyl radicals having one or more alkoxy radicals attached to the alkyl radical, that is, to form monoalkoxyalkyl and dialkoxyalkyl radicals. Examples of such radicals include methoxy, ethoxy, propoxy, butoxy and *tert*-butoxy alkyls. The "alkoxy" radicals may be further substituted with one or more halo atoms, such as fluoro, chloro or bromo, to provide "haloalkoxy" radicals. Examples of such radicals include fluoromethoxy, chloromethoxy, trifluoromethoxy, difluoromethoxy, trifluoroethoxy, fluoroethoxy, tetrafluoroethoxy, pentafluoroethoxy, and fluoropropoxy.

The term "alkylthio" embraces radicals containing a linear or branched alkyl radical, of 1 to about 6 carbon atoms, attached to a divalent sulfur atom. An example of "lower alkylthio" is methylthio (CH_3-S-).

The term "alkylthioalkyl" embraces alkylthio radicals, attached to an alkyl group. Examples of such radicals include methylthiomethyl.

The term "halo" means halogens such as fluorine, chlorine, bromine or iodine atoms.

The term "heterocyclyl" means a saturated or unsaturated mono- or multi-ring carbocycle wherein one or more carbon atoms is replaced by N, S, P, or O. This includes, for example, the following structures:



wherein Z, Z^1, Z^2 or Z^3 is C, S, P, O, or N, with the proviso that one of Z, Z^1, Z^2 or Z^3 is other than carbon, but is not O or S when attached to another Z atom by a double bond or when attached to another O or S atom. Furthermore, the optional substituents are understood to be attached to Z, Z^1, Z^2 or Z^3 only when each is C. The term "heterocyclyl" also includes fully saturated ring structures such as piperazinyl, dioxanyl, tetrahydrofuranyl, oxiranyl, aziridinyl, morpholinyl, pyrrolidinyl, piperidinyl, thiazolidinyl, and others. The term "heterocyclyl" also includes partially unsaturated ring structures such as dihydrofuranyl, pyrazolinyl, imidazolinyl, pyrrolinyl, chromanyl, dihydrothiophenyl, and others.

The term "heteroaryl" means a fully unsaturated heterocycle.

In either "heterocycle" or "heteroaryl," the point of attachment to the molecule of interest can be at the heteroatom or elsewhere within the ring.

The term "cycloalkyl" means a mono- or multi-ringed carbocycle wherein each ring contains three to about seven carbon atoms, preferably three to about five carbon atoms. Examples include radicals such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloalkenyl, and cycloheptyl. The term "cycloalkyl" additionally encompasses spiro systems wherein the cycloalkyl ring has a carbon ring atom in common with the seven-membered heterocyclic ring of the benzothiepine.

The term "oxo" means a doubly bonded oxygen.

The term "alkoxy" means a radical comprising an alkyl radical that is bonded to an oxygen atom, such as a methoxy radical. More preferred alkoxy radicals are "lower alkoxy" radicals having one to about ten carbon atoms. Still more preferred alkoxy radicals have one to about six carbon atoms. Examples of such radicals include methoxy, ethoxy, propoxy, isopropoxy, butoxy and tert-butoxy.

The term "aryl" means a fully unsaturated mono- or multi-ring carbocycle, including, but not limited to, substituted or unsubstituted phenyl, naphthyl, or anthracenyl.

The phrase "optionally substituted" means that the indicated radical may, but need not be substituted for hydrogen. Thus, the phrase "optionally substituted by one or more" means that if a substitution is made at the indicated moiety, more than one substitution is contemplated as well. In this regard, if more than one optional substituent exists, either substituent may be selected, or a combination of substituents may be selected, or more than one of the same substituent may be selected. By way of example, and not limitation, the phrase "C_1-C_5 alkyl optionally substituted by one or more halo or alkoxy" should be taken to mean, for example, that methyl, ethyl, propyl, butyl, or pentyl may have at all substitutable positions: hydrogen, fluorine, chlorine or other halogen, methoxy, ethoxy, propoxy, *iso* butoxy, *tert*-butoxy, pentoxy or other alkoxy radicals, and combinations thereof. Non-limiting examples include: propyl, *iso*-propyl, methoxypropyl, fluoromethyl, fluoropropyl, 1-fluoro-methoxymethyl and the like.

When a compound is described by both a structure and a name, the name is intended to correspond to the indicated structure, and similarly the structure is intended to correspond with the indicated name.

The term "subject" as used herein refers to an animal, in one embodiment a mammal, and in an exemplary embodiment particularly a human being, who is the object of treatment, observation or experiment.

The terms "dosing" and "treatment" as used herein refer to any process, action, application, therapy or the like, wherein a subject, particularly a human being, is rendered medical aid with the object of improving the subject's condition, either directly or indirectly.

The term "therapeutic compound" as used herein refers to a compound useful in the prevention or treatment of an inflammatory condition or disease of the gastrointestinal tract.

The term "combination therapy" means the administration of two or more therapeutic compounds to treat a therapeutic condition or disorder described in the present disclosure, for example inflammatory bowel disease including Crohn's disease and ulcerative colitis, peptic ulcer disease including gastric ulceration, duodenal ulceration and esophageal ulceration, gastroesophageal reflux disease, irritable bowel syndrome, and other inflammatory conditions including gastritis, ileitis, esophagitis, paralytic ileus and diarrhea. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients or in multiple, separate capsules for each active ingredient. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

The term "therapeutic combination" as used herein refers to the combination of the two or more therapeutic compounds and to any pharmaceutically acceptable carriers used to provide dosage forms that produce a beneficial effect of each therapeutic compound in the subject at the desired time, whether the therapeutic compounds are administered substantially simultaneously, or sequentially.

The term "therapeutically effective" as used herein refers to a characteristic of an amount of a therapeutic compound, or a characteristic of amounts of combined therapeutic compounds in combination therapy. The amount or combined amounts achieve the goal of preventing, avoiding, reducing or eliminating the inflammatory condition or disease of the gastrointestinal tract.

The terms "inducible nitric oxide synthase" and "iNOS" as used interchangeably herein refer to the Ca^{+2} –independent, inducible isoform of the enzyme nitric oxide synthase.

The terms "inducible nitric oxide synthase selective inhibitor", "selective iNOS inhibitor" and "iNOS selective inhibitor" as used interchangeably herein refer to a therapeutic compound that selectively inhibits the Ca^{+2} –independent, inducible isoform of the enzyme nitric oxide synthase. A selective iNOS inhibitor is defined as producing the selective inhibition of iNOS compared to either endothelial NOS or neuronal NOS such that in vivo administration results in efficacy (ED_{50} less than 100 mg/kg, but preferably less than 10 mg/kg in a rodent endotoxin model) and selectivity of at least 20-fold, but preferably 100-fold or greater with respect to eNOS as measured by elevation in mean arterial blood pressure and selectivity of at least 20-fold, but preferably 100-fold or greater with respect to nNOS as measured by reductions in gastrointestinal transit or penile erection.

The term "prodrug" refers to a compound that is a drug precursor which, following administration to a subject and subsequent absorption, is converted to an active species in vivo via some process, such as a metabolic process. Other products from the conversion process are easily disposed of by the body. The more preferred prodrugs are those involving a conversion process that produces products that are generally accepted as safe.

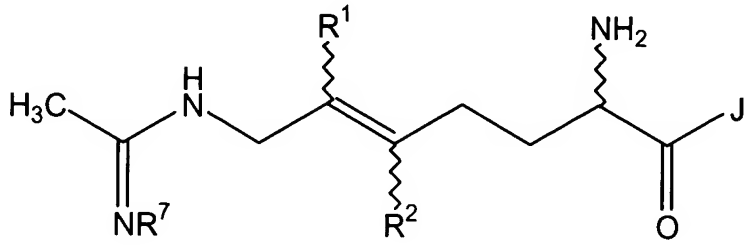
The term "gastrointestinal tract" refers to the esophagus, stomach, and small and large intestines including the duodenum, ileum and colon. Inflammatory conditions of the gastrointestinal tract include inflammatory bowel disease including Crohn's disease and ulcerative colitis, peptic ulcer disease including gastric ulceration, duodenal ulceration and esophageal ulceration, gastroesophageal reflux disease, irritable bowel syndrome and other chronic inflammatory conditions including gastritis, ileitis, colitis, esophagitis, paralytic ileus and diarrhea.

The term "anti-inflammatory effective" as used herein refers to a characteristic of an amount of a therapeutic compound, or a characteristic of amounts of combined therapeutic compounds in combination therapy. The amount or combined amounts achieve the goal of preventing, avoiding, reducing or eliminating inflammation.

The term "anti-microbial" as used herein refers to the characteristic of a compound or agent as useful in reducing or eliminating infection by a microbe including a bacterium, and particularly infection by the bacterium *H. pylori*, or in strengthening mucosal defenses of the stomach and duodenum against such microbial infection. Anti-microbials include antibiotics, cytoprotective agents or compounds such as bismuth compounds in the form of bismuth subsalicylate and colloidal bismuth subcitrate, sucralfate and carbenoxalone. Thus, antimicrobial agents useful in the present invention include for example, a nitroimidazole, a proton-pump inhibitor, a bismuth compound, or any antibiotic compound such as penicillin. More specifically, antimicrobial compounds useful in combination with a selective iNOS inhibitor according to the methods of the present invention include amoxicillin, clarithromycin, rifabutin, bismuth subsalicylate, metronidazole, omeprarazole, ranitidine, and tetracycline, alone or in combination with one another. A double anti-microbial compound useful in the methods of the present invention is, for example, a combination of omeprazole and amoxicillin. A triple anti-microbial compound useful in the methods of the present invention is, for example, a combination of ranitidine, metronidazole, and amoxicillin.

The term "anti-secretory" refers to any compound or agent useful in inhibiting the secretion of gastric acid including H_2 histamine receptor antagonists and proton pump inhibitors. H_2 histamine receptor antagonists include burimamide, cimetidine, ranitidine, famotidine and nizatidine. Proton pump inhibitors, i.e. specific inhibitors of the H^+,K+-ATP-ase, include the substituted benzimidazole compounds lansoprazole and omeprazole.

In one illustrative example of a selective iNOS inhibitor useful in the methods of the present invetnion, treatment is facilitated through compounds having Formula I:



I

or a pharmaceutically acceptable salt thereof, wherein:

R^1 is selected from the group consisting of H, halo and alkyl which may be optionally substituted by one or more halo;

R^2 is selected from the group consisting of H, halo and alkyl which may be optionally substituted by one or more halo;

with the proviso that at least one of R^1 or R^2 contains a halo;

R^7 is selected from the group consisting of H and hydroxy; and

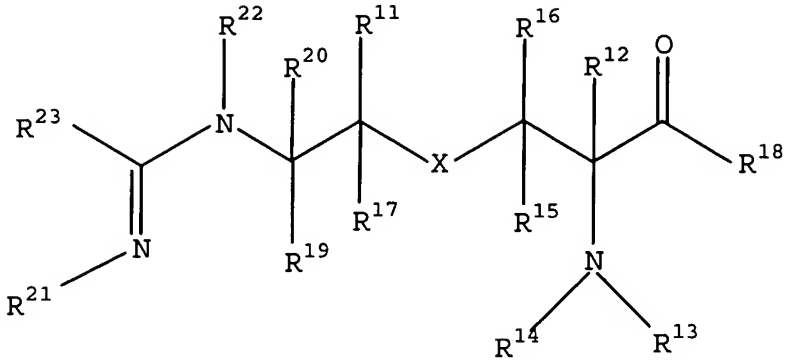
J is selected from the group consisting of hydroxy, alkoxy, and NR^3R^4 wherein;

R^3 is selected from the group consisting of H, lower alkyl, lower alkylenyl and lower alkynyl; and

R^4 is selected from the group consisting of H, and a heterocyclic ring in which at least one member of the ring is carbon and in which 1 to about 4 heteroatoms are independently selected from oxygen, nitrogen and sulfur and said heterocyclic ring may be optionally substituted with heteroarylamino, N-aryl-N-alkylamino, N-heteroarylamino-N-alkylamino, haloalkylthio, alkanoyloxy, alkoxy, heteroaralkoxy, cycloalkoxy, cycloalkenyloxy, hydroxy, amino, thio, nitro, lower alkylamino, alkylthio, alkylthioalkyl, arylamino, aralkylamino, arylthio, alkylsulfinyl, alkylsulfonyl, alkylsulfonamido, alkylaminosulfonyl,

amidosulfonyl, monoalkyl amidosulfonyl, dialkyl amidosulfonyl, monoarylamidosulfonyl, arylsulfonamido, diarylamidosulfonyl, monoalkyl monoaryl amidosulfonyl, arylsulfinyl, arylsulfonyl, heteroarylthio, heteroarylsulfinyl, heteroarylsulfonyl, alkanoyl, alkenoyl, aroyl, heteroaroyl, aralkanoyl, heteroaralkanoyl, haloalkanoyl, alkyl, alkenyl, alkynyl, alkylenedioxy, haloalkylenedioxy, cycloalkyl, cycloalkenyl, lower cycloalkylalkyl, lower cycloalkenylalkyl, halo, haloalkyl, haloalkoxy, hydroxyhaloalkyl, hydroxyaralkyl, hydroxyalkyl, hydoxyheteroaralkyl, haloalkoxyalkyl, aryl, aralkyl, aryloxy, aralkoxy, aryloxyalkyl, saturated heterocyclyl, partially saturated heterocyclyl, heteroaryl, heteroaryloxy, heteroaryloxyalkyl, arylalkyl, heteroarylalkyl, arylalkenyl, heteroarylalkenyl, cyanoalkyl, dicyanoalkyl, carboxamidoalkyl, dicarboxamidoalkyl, cyanocarboalkoxyalkyl, carboalkoxyalkyl, dicarboalkoxyalkyl, cyanocycloalkyl, dicyanocycloalkyl, carboxamidocycloalkyl, dicarboxamidocycloalkyl, carboalkoxycyanocycloalkyl, carboalkoxycycloalkyl, dicarboalkoxycycloalkyl, formylalkyl, acylalkyl, dialkoxyphosphonoalkyl, diaralkoxyphosphonoalkyl, phosphonoalkyl, dialkoxyphosphonoalkoxy, diaralkoxyphosphonoalkoxy, phosphonoalkoxy, dialkoxyphosphonoalkylamino, diaralkoxyphosphonoalkylamino, phosphonoalkylamino, dialkoxyphosphonoalkyl, diaralkoxyphosphonoalkyl, guanidino, amidino, and acylamino.

In another embodiment, the present invention provides treatment utilizing a compound or a salt thereof, the compound having a structure corresponding to Formula **II**:



II

In the structure of Formula II, X is selected from the group consisting of -S-, -S(O)-, and -$S(O)_2$-. Preferably, X is -S-. R^{12} is selected from the group consisting of C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, C_1-C_5 alkoxy-C_1 alkyl, and C_1-C_5 alkylthio-C_1 alkyl wherein each of these groups is optionally substituted by one or more substituent selected from the group consisting of -OH, alkoxy, and halogen. Preferably, R^{12} is C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH, alkoxy, and halogen. With respect to R^{13} and R^{18}, R^{18} is selected from the group consisting of $-OR^{24}$ and $-N(R^{25})(R^{26})$, and R^{13} is selected from the group consisting of -H, -OH, $-C(O)-R^{27}$, $-C(O)-O-R^{28}$, and $-C(O)-S-R^{29}$; or R^{18} is $-N(R^{30})-$, and R^{13} is -C(O)-, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring; or R^{18} is -O-, and R^{13} is $-C(R^{31})(R^{32})-$, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring. If R^{13} is $-C(R3^{21})(R^{32})-$, then R^{14} is $-C(O)-O-R^{33}$; otherwise R^{14} is -H. R^{11}, R^{15}, R^{16}, and R^{17} independently are selected from the group consisting of -H, halogen, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_5 alkoxy-C_1 alkyl. R^{19} and R^{20} independently are selected from the group consisting of -H, C_1-C_6 alkyl, C_2-C_6 alkenyl, C_2-C_6 alkynyl, and C_1-C_5 alkoxy-C_1 alkyl. With respect to R^{21} and R^{22}, R^{21} is selected from the group consisting of -H, -OH, $-C(O)-O-R^{34}$, and $-C(O)-S-R^{35}$, and R^{22} is selected from the group consisting of -H, -OH, $-C(O)-O-R^{36}$, and $-C(O)-S-R^{37}$; or R^{21} is -O-, and R^{22} is -C(O)-, wherein R^{21} and R^{22} together with the atoms to

which they are attached form a ring; or R^{21} is -C(O)-, and R^{22} is -O-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring. R^{23} is C_1 alkyl. R^{24} is selected from the group consisting of -H and C_1-C_6 alkyl, wherein when R^{24} is C_1-C_6 alkyl, R^{24} is optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. With respect to R^{25} and R^{26}, R^{25} is selected from the group consisting of -H, alkyl, and alkoxy, and R^{26} is selected from the group consisting of -H, -OH, alkyl, alkoxy, -C(O)-R^{38}, -C(O)-O-R^{39}, and -C(O)-S-R^{40}; wherein when R^{25} and R^{26} independently are alkyl or alkoxy, R^{25} and R^{26} independently are optionally substituted with one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl; or R^{25} is -H; and R^{26} is selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35}, R^{36}, R^{37}, R^{38}, R^{39}, and R^{40} independently are selected from the group consisting of -H and alkyl, wherein alkyl is optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl. When any of R^{11}, R^{12}, R^{13}, R^{14}, R^{15}, R^{16}, R^{17}, R^{18}, $R19^{9}$, R^{20}, R^{21}, R^{22}, R^{23}, R^{24}, R^{25}, R^{26}, R^{27}, R^{28}, R^{29}, R^{30}, R^{31}, R^{32}, R^{33}, R^{34}, R^{35} R^{36}, R^{37}, R^{38}, R^{39}, and R^{40} independently is a moiety selected from the group consisting of alkyl, alkenyl, alkynyl, alkoxy, alkylthio, cycloalkyl, heterocyclyl, aryl, and heteroaryl, then the moiety is optionally substituted by one or more substituent selected from the group consisting of -OH, alkoxy, and halogen.

In a preferred compound, R^{18} is -OH. When R^{18} is -OH, preferably X is S. In a further compound, R^{11}, R^{15}, R^{16}, R^{17}, R^{19}, and R^{20} independently are selected from the group consisting of -H and C_1-C_3 alkyl. Preferably R^{15}, R^{16}, R^{17}, R^{19}, R^{20} each are -H. R^{23} can be a variety of groups, for example fluoromethyl or methyl. R^{11} can be C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH and halogen; preferably R^{11} is C_1 alkyl optionally substituted with halogen; more preferably R^{11} is

selected from the group consisting of fluoromethyl, hydroxymethyl, and methyl. In one important compound, R^{11} can be methyl. Alternatively, R^{11} can be fluoromethyl. In another alternative R^{11} can be hydroxymethyl. In another compound, R^{12} is C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH, alkoxy, and halogen. In one preferred compound R^{12} is C_1 alkyl optionally substituted with halogen. For example, R^{12} can be methyl. Alternatively, R^{12} can be fluoromethyl. In yet another example, R^{12} can be hydroxymethyl. In still another example, R^{12} can be methoxymethyl.

In this exemplary compound, it is preferred that R^{13}, R^{14}, R^{21} and R^{22} each is -H. In this compound, it is further preferred that R^{11}, R^{15}, R^{16}, R^{17}, R^{19}, and R^{20} independently are selected from the group consisting of -H and C_1-C_3 alkyl. Preferably R^{15}, R^{16}, R^{17}, R^{19}, R^{20} each is -H. In this further compound, R^{23} can be, for example, fluoromethyl, or in another example R^{23} can be methyl. In preferred compounds of these examples, R^{12} is C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH, alkoxy, and halogen. Preferably R^{12} is C_1 alkyl optionally substituted with halogen. In one such example R^{12} is fluoromethyl. In another example R^{12} is methyl. Alternatively R^{12} can be hydroxymethyl. In another alternative, R^{12} can be methoxymethyl.

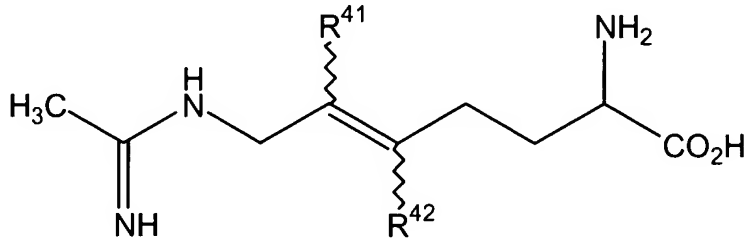
When R^{23} is methyl, R^{11} can be, for example, -H or C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH and halogen. In a preferred compound R^{11} is -H. Alternatively, R^{11} can be C_1-C_6 alkyl optionally substituted with a substituent selected from the group consisting of -OH and halogen. For example R^{11} can be methyl, ethyl, n-propyl, i-propyl, n-butyl, sec-butyl, isobutyl, t-butyl, a pentyl isomer, or a hexyl isomer. For example, R^{11} can be ethyl. Alternatively, R^{11} can be C_1 alkyl optionally substituted with a substituent selected from the group consisting of -OH and halogen; for example R^{11} can be methyl. Alternatively, R^{11} can be fluoromethyl. In another alternative, R^{11} can be hydroxymethyl.

In another compound R^{18} can be $-OR^{24}$. R^{24} can be as defined above. Preferably R^{24} is C_1-C_6 alkyl optionally substituted by one or more moieties selected from the group consisting of cycloalkyl, heterocyclyl, aryl, and heteroaryl; more preferably R^{24} is C_1-C_3 alkyl; and more preferably still R^{24} is methyl. In yet another example of compound **II**, R^{18} can be $-N(R^{25})(R^{26})$, wherein R^{25} and R^{26} are as defined above. In still another compound, R^{18} can be $-N(R^{30})-$, and R^{13} can be -C(O)-, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring. In another example still, R^{18} can be -O-, and R^{13} can be $-C(R^{31})(R^{32})-$, wherein R^{18} and R^{13} together with the atoms to which they are attached form a ring.

In a compound of Formula **II**, R^{21} can be selected from the group consisting of -OH, $-C(O)-O-R^{34}$, and $-C(O)-S-R^{35}$. Preferably R^{21} is -OH. In a further example, R^{22} is -H when R^{21} is -OH.

However, the present example also provides useful compounds of Formula **II** in which R^{21} is -O-, and R^{22} is -C(O)-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring. In another useful compound, R^{21} is -C(O)-, and R^{22} is -O-, wherein R^{21} and R^{22} together with the atoms to which they are attached form a ring. Alternatively, R^{22} can be selected from the group consisting of -OH, $-C(O)-O-R^{36}$, and $-C(O)-S-R^{37}$. In this alternative, R^{21} is preferably -H.

In another selective iNOS inhibitor useful in the practice of the present invention, a compound is represented by Formula **III**:



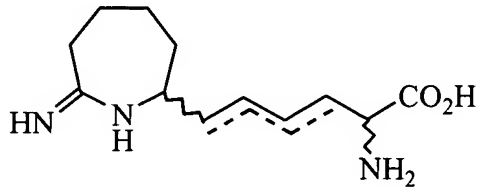
III

or a pharmaceutically acceptable salt thereof, wherein:

R^{41} is H or methyl; and

R^{42} is H or methyl.

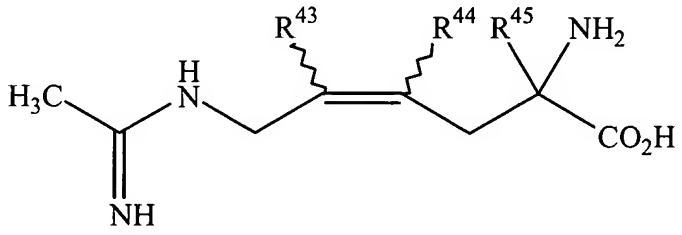
Another selective iNOS inhibitor useful in the practice of the present invention is represented by a compound of formula **IV**



IV

or a pharmaceutically acceptable salt thereof.

Another exemplary selective iNOS inhibitor useful in the present invention is represented by Formula **V**:



V

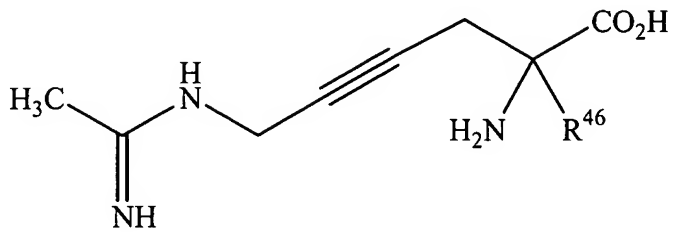
or a pharmaceutically acceptable salt thereof, wherein:

R^{43} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{44} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{45} is C_1-C_5 alkyl or C_1-C_5 alkyl be substituted by alkoxy or one or more halo.

A further illustrative selective iNOS inhibitor is represented by Formula **VI**:

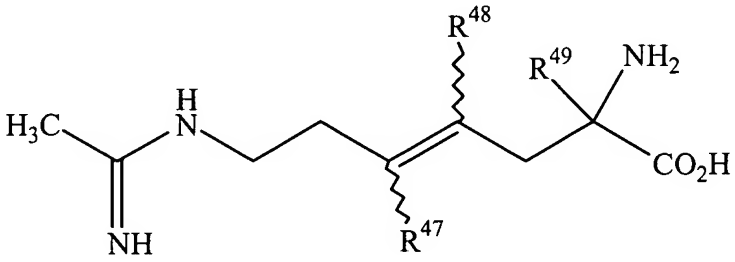


VI

or a pharmaceutically acceptable salt thereof, wherein:

R^{46} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo.

Another exemplary selective iNOS inhibitor useful in the present invention is represented by Formula **VII**



VII

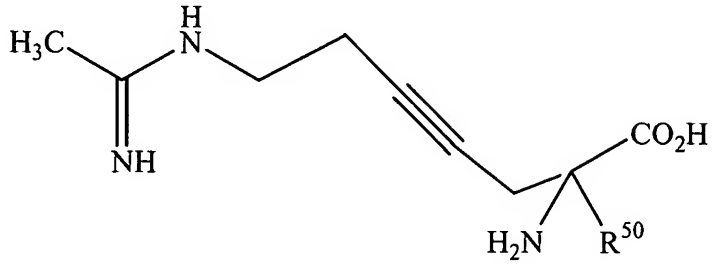
or a pharmaceutically acceptable salt thereof, wherein:

R^{47} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{48} is selected from the group consisting of hydrogen, halo, C_1-C_5 alkyl and C_1-C_5 alkyl substituted by alkoxy or one or more halo;

R^{49} is C_1-C_5 alkyl or C_1-C_5 alkyl be substituted by alkoxy or one or more halo.

Another exemplary selective iNOS inhibitor useful in the present invention is represented by Formula **VIII**

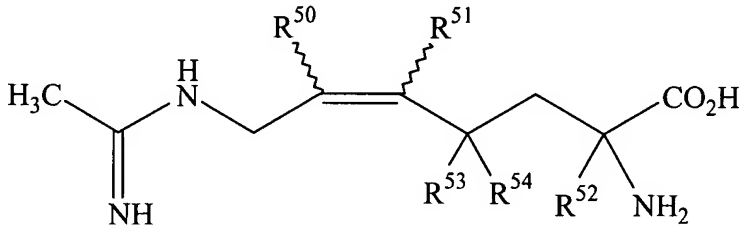


VIII

or a pharmaceutically acceptable salt thereof, wherein:

R^{50} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo.

Another selective iNOS inhibitor useful in the practice of the present invention is represented by a compound of formula **IX**



IX

or a pharmaceutically acceptable salt thereof, wherein:

R^{50} is selected from the group consisting of hydrogen, halo, and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

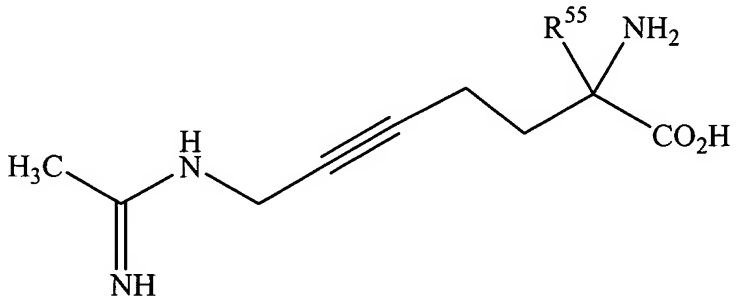
R^{51} is selected from the group consisting of hydrogen, halo, and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

R^{52} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo;

R^{53} is selected from the group consisting of hydrogen, halo, andC_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo; and

R^{54} is selected from the group consisting of halo and C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo.

Yet another selective iNOS inhibitor useful in the practice of the present invention is represented by a compound of formula **X**

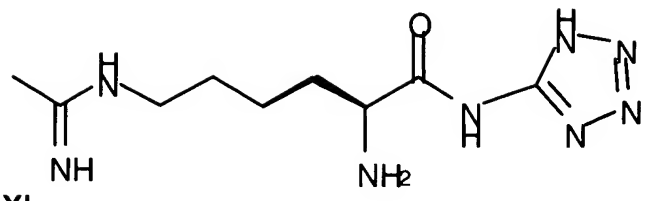


X

or a pharmaceutically acceptable salt thereof, wherein:

R^{55} is C_1-C_5 alkyl, said C_1-C_5 alkyl optionally substituted by halo or alkoxy, said alkoxy optionally substituted by one or more halo.

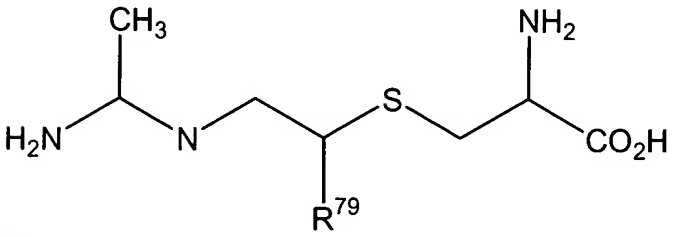
In another exemplary compound, the inducible nitric oxide synthase selective inhibitor is the compound having the formula XI, or a pharmaceutically acceptable thereof. Compound XI has previously been described in International Publication Number WO 00/26195, published May 11, 2000, which is herein incorporated by reference.



XI

2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazol-5-yl) hexanamide, hydrate, dihydrochloride

The invention also contemplates use of other selective iNOS inhibitors. By way of example, iNOS selective inhibitors also useful in the present invention are described in U.S. Patent No. 6,355,689, Beswick et al., filed November 29, 2000 and issued March 12, 2002, which describes and claims a selective iNOS inhibitor with the formula XII:



XII

wherein R^{79} is selected from C_{1-4} alkyl, C_{3-4} cycloalkyl, C_{1-4} hydroxyalkyl, and C_{1-4} haloalkyl. The description of U.S. Patent 6,355,689 states that R^{79} is preferably C_{1-4} alkyl, and most preferably, methyl. Specific embodiments disclosed in US Patent 6,355,689 and suitable for use in the present methods and compositions include:

S-((R)-2-(1-iminoethylamino)propyl)-L-cysteine;

S-((S)-2-(1-iminoethylamino)propyl)-L-cysteine;

S-((R/S)-2-(1-iminoethylamino)propyl)-L-cysteine;

S-((R)-2-(1-iminoethylamino)propyl)-D-cysteine;

S-((S)-2-(1-iminoethylamino)propyl)-D-cysteine;

S-((R/S)-2-(1-iminoethylamino)propyl)-D-cysteine;

S-((R/S)-2-(1-iminoethylamino)butyl)-L-cysteine;

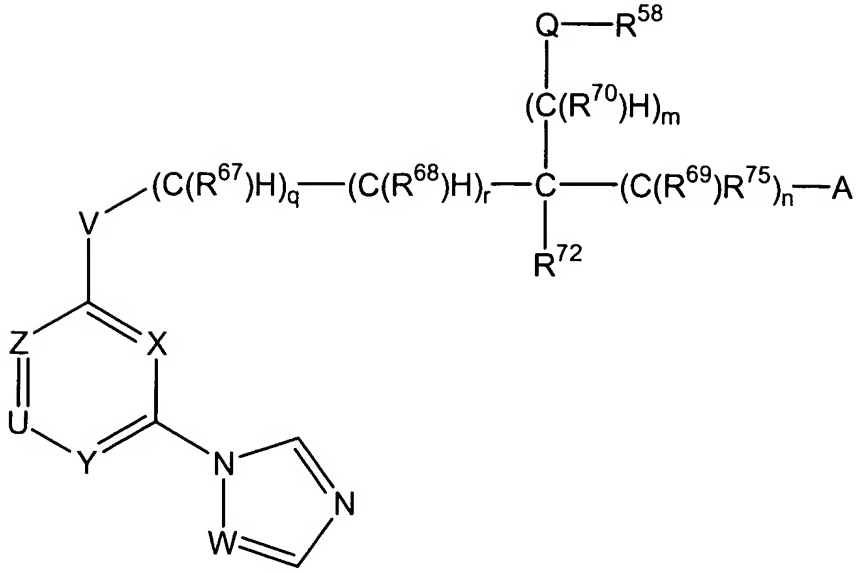
S-((R/S)-2-(1-iminoethylamino,2-cyclopropyl)ethyl)-L-cysteine; and

S-((R/S)-2-(1-iminoethylamino,3-hydroxy)propyl)-L-cysteine,

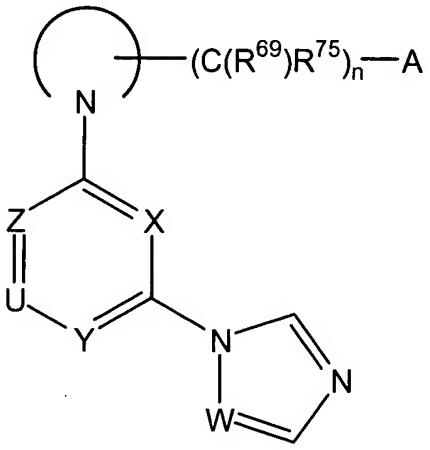
or a pharmaceutically acceptable salt, solvate, or physiologically functional derivative thereof.

The above selective iNOS inhibitors are believed to work by competing with arginine as a substrate for the iNOS enzyme. Another strategy for inhibition of iNOS has been described by Arnaiz et al. in international patent application number PCT/US98/03176, publication number WO 98/37079 (Berlex Laboratories, Inc. Richmond, CA 94804-0099 and Pharmacopeia, Inc. Princeton, NJ 08540), published August 27, 1998 (Arnaiz). The Arnaiz application describes inhibitors of iNOS monomer dimerization. The iNOS enzyme is a homodimer; each monomer has a reductase domain, incorporating binding sites for flavin cofactors (FAD and FMN) and for NADPH. The reductase domain supplies electrons to the oxidase domain of the other monomer, where L-arginine is oxidized at the active site, which incorporates a heme group (Fe) cytochrome P-450 domain. Tetrahydrobiopterin (BH4) is required for homodimerization and modulates the heme redox state during electron transfer. iNOS monomers are inactive, and dimerization is required for activity.

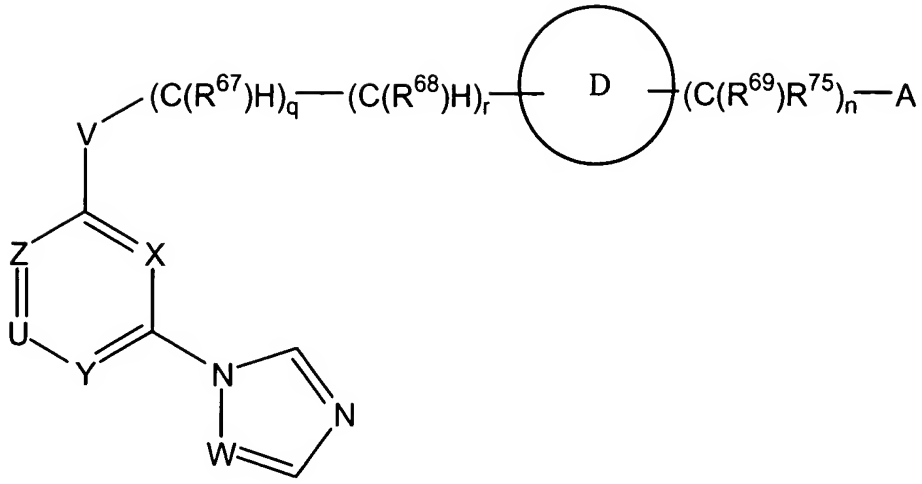
Thus, in another embodiment of the present invention, the selective iNOS inhibitor is a dimerization inhibitor represented by a compound of Formula XIII, Formula XIV or Formula XV:



Formula XIII;



Formula XIV; or



Formula XV;

wherein:

A is $-R^{56}$, $-OR^{56}$, $C(O)N(R^{56})R^{57}$, $P(O)[N(R^{56})R^{57}]_2$, $-N(R^{56})C(O)R^{57}$, $-N(R^{76})C(O)OR^{56}$, $-N(R^{56})R^{76}$, $-N(R^{71})C(O)N(R^{56})R^{71}$, $-S(O)_tR^{56}$, $-SO_2NHC(O)R^{56}$, $-NHSO_2R^{77}$, $-SO_2NH(R^{56})H$, $-C(O)NHSO_2R^{77}$, and $-CH=NOR^{56}$;

each X, Y and Z are independently N or $C(R^{19})$;

each U is N or $C(R^{60})$, provided that U is N only when X is N and Z and Y are CR^{74};

V is $N(R^{59})$, S, O or $C(R^{59})H$;

Each W is N or CH;

Q is chosen from the group consisting of a direct bond, -C(O)-, -O-, $-C(=N-R^{56})-$, $S(O)_t$, and $-N(R^{61})-$;

m is zero or an integer from 1 to 4;

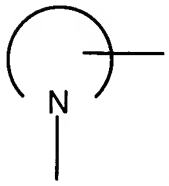
n is zero or an integer from 1 to 3;

q is zero or one;

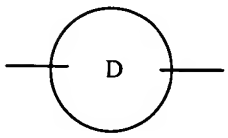
r is zero or one, provided that when Q and V are heteroatoms, m, q, and r cannot all be zero;

when A is $-OR^{56}$, $N(R^{56})C(O)R^{57}$, $-N(R^{71})C(O)OR^{57}$, $-N(R^{56})R^{76}$, $-N(R^{71})C(O)N(R^{56})R^{71}$, $-S(O)_tR^{56}$ (where t is zero), or $-NHSO_2R^{77}$, n, q, and r cannot all be zero; and when Q is a heteroatom and A is $-OR^{56}$, $N(R^{56})C(O)R^{57}$, $-N(R^{71})C(O)OR^{57}$, $-N(R^{56})R^{76}$, $N(R^{71})C(O)N(R^{56})R^{71}$, $-S(O)_tR^{56}$ (when t is zero), or $-NHSO_2R^{77}$, m and n cannot both be zero;

t is zero, one or two;



is an optionally substituted N-heterocyclyl;



is an optionally substituted carbocyclyl or optionally substituted N-heterocyclyl;

each R^{56} and R^{57} are independently chosen from the group consisting of hydrogen, optionally substituted C_1-C_{20} alkyl, optionally substituted cycloalkyl, -[C_0-C_8 alkyl]-R^{64}, -[C_2-C_8 alkenyl]-R^{64}, -[C_2-C_8 alkynyl]-R^{64}, -[C_2-C_8 alkyl]-R^{65} (optionally substituted by hydroxy), -[C_1-C_8]-R^{66} (optionally substituted by hydroxy), optionally substituted heterocyclyl;

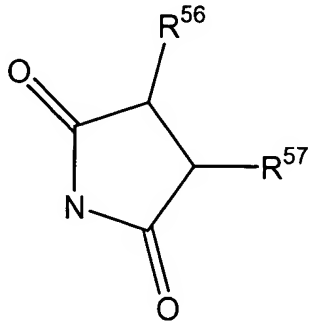
or R^{56} and R^{57} together with the nitrogen atom to which they are attached is an optionally substituted N-heterocyclyl;

R^{58} is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, optionally substituted aryl, haloalkyl, -[C_1-C_8 alkyl]-$C(O)N(R^{56})R^{57}$, -[C_1-C_8 alkyl]- $N(R^{56})R^{57}$, -[C_1-C_8 alkyl]-R^{63}, -[C_2-C_8 alk2yl]-R^{65}, -[C_1-C_8 alkyl]-R^{66}, and heterocyclyl (optionally substituted by one or more substitutents selected from the group consisting of halo, alkyl, alkoxy and imidazolyl);

or when Q is $-N(R^{58})-$ or a direct bond to R^{58}, R^{58} may additionally be aminocarbonyl,

alkoxycarbonyl, alkylsulfonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl and $-C(=NR^{73})-NH_2$;

or $-Q-R^{58}$ taken together represents $-C(O)OH$, $-C(O)N(R^{56})R^{57}$ or



;

R^{59} is chosen from the group consisting of hydrogen, alkyl, aryl, aralkyl and cycloalkyl;

Provided that when A is $-R^{56}$ or $-OR^{56}$, R^{59} cannot be hydrogen, and when V is CH, R^{59} may additionally be hydroxy;

R^{60} is chosen from the group consisting of hydrogen, alkyl, aryl, aralkyl, haloalkyl,

optionally substituted aralkyl, optionally substituted aryl, $-OR^{71}$, $-S(O)_t-R^{71}$, $N(R^{71})R^{76}$, $N(R^{71})C(O)N(R^{56})R^{71}$, $N(R^{71})C(O)OR^{71}$, $N(R^{71})C(O)\ R^{71}$, $-[C_0-C_8\ alkyl]-C(H)[C(O)R^{71}]_2$ and $-[C_0-C_8\ alkyl]-\ C(O)N(R^{56})R^{71}$;

R^{61} is chosen from the group consisting of hydrogen, alkyl, cycloalkyl, $-[C_1-C_8\ alkyl]-R^{63}$, $-[C_2-C_8]alkyl]-R^{65}$, $-[C_1-C_8\ alkyl]-R^{66}$, acyl, $-C(O)R^{63}$, $-C(O)-\ -[C_1-C_8\ alkyl]-R^{63}$, alkoxycarbonyl, optionally substituted aryloxycarbonyl, optionally substituted aralkoxycarbonyl, alkylsulfonyl, optionally substituted aryl, optionally substituted heterocyclyl, alkoxycarbonylalkyl, carboxyalkyl, optionally substituted arylsulfonyl, aminocarbonyl, monoalkylaminocarbonyl, dialkylaminocarbonyl, optionally substituted arylaminocarbonyl, aminosulfonyl,

monoalkylaminosulfonyl dialkylaminosulfonyl, arylaminosulfonyl, arylsulfonylaminocarbonyl, optionally substituted N-heterocyclyl, -C(=NH)-N(CN)R^{56}, -C(O)R^{78}-N(R^{56})R^{57}, -C(O)-N(R^{56})R^{78}-C(O)OR^{56};

each R^{63} and R^{64} are independently chosen from the group consisting of haloalkyl,

cycloalkyl, (optionally substituted with halo, cyano, alkyl or alkoxy), carbocyclyl (optionally substituted with one or more substituents selected from the group consisting of halo, alkyl and alkoxy) and heterocyclyl (optionally substituted with alkyl, aralkyl or alkoxy);

each R^{65} is independently chosen from the group consisting of halo, alkoxy, optionally

substituted aryloxy, optionally substituted aralkoxy, optionally substituted –$S(O)_t$-R^{77}, acylamino, amino, monoalkylamino, dialkylamino, (triphenylmethyl)amino, hydroxy, mercapto, alkylsulfonamido;

each R^{66} is independently chosen from the group consisting of cyano, di(alkoxy)alkyl,

carboxy, alkoxycarbonyl, aminocarbonyl, monoalkylaminocarbonyl and dialkylaminocarbonyl;

each R^{67}, R^{68}, R^{69}, R^{70}, R^{72}, and R^{75} are independently hydrogen or alkyl;

each R^{71} is independently hydrogen, alkyl, optionally substituted aryl, optionally substituted aralkyl or cycloalkyl;

R^{73} is hydrogen, NO_2, or toluenesulfonyl;

each R^{74} is independently hydrogen, alkyl (optionally substituted with hydroxy), cyclopropyl, halo or haloalkyl;

each R^{76} is independently hydrogen, alkyl, cycloalkyl, optionally substituted aryl,

optionally substituted aralkyl, -C(O)R^{77} or –SO_2R^{77};

or R^{76} taken together with R^{56} and the nitrogen to which they are attached is an optionally

substituted N-heterocyclyl;

or R^{76} taken together with R^{71} and the nitrogen to which they are attached is an optionally

substituted N-heterocyclyl;

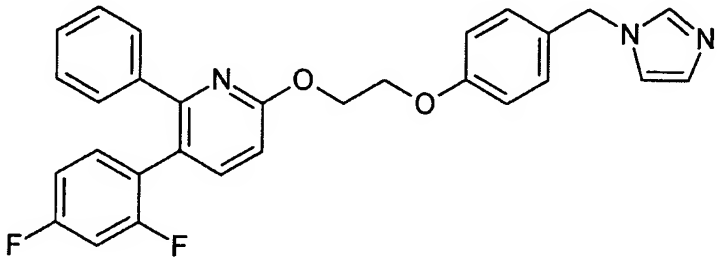
each R^{77} is independently alkyl, cycloalkyl, optionally substituted aryl or optionally

substituted aralkyl; and

R^{78} is an amino acid residue;

as a single stereoisomer or mixture thereof, or a pharmaceutically acceptable salt thereof.

Another iNOS dimerization inhibitor, 3-(2,4-difluorophenyl)-6-{2-[4-(1*H*-imidazol-1-ylmethyl) phenoxy]ethoxy}-2-phenylpyridine (PPA250) has been described in Ohtsuka et al., *J Phamacol Exp Ther* Vol. 303, Issue 1, 52-57, October 2002. PPA250 has the structure:



PPA250

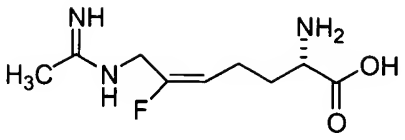
Therefore, in another embodiment of the present invention, the compound PPA250 may be employed as the selective iNOS inhibitor.

b. Illustrative Examples

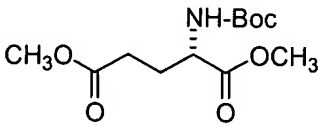
The following synthesis examples are shown for illustrative purposes and in no way intended to limit the scope of the invention. Where isomers are

not defined, utilization of appropriate chromatography methods will afford single isomers.

Example A

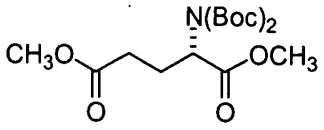


(2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride, monohydrate

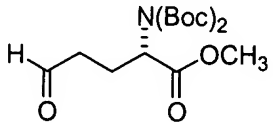


EX-A-1) Trimethylsilyl chloride (107.8 g, 1.00 mol) was added dropwise to a cooled solution of L-glutamic acid (30.00 g, 0.20 mol) in 300 mL of methanol at 0 °C. The resulting clear, colorless solution was allowed to stir at room temperature. After 18 h, analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The reaction was then cooled to 0 °C, triethylamine (134 g, 1.33 mol) was added, and a white precipitate formed. Di-tert-butyldicarbonate (49 g, 0.23 mol) was added, and the mixture was allowed to warm to room temperature. After 3 h the solvent was removed, and 700 mL of diethyl ether was added. The solution was filtered, and the filter cake was rinsed with an additional 500 mL of diethyl ether. The filtrate was concentrated to 60.8 g (>95%) of a tan oil which was carried onto the next step without further purification. LCMS: *m/z* = 298.1

$[M+Na]^+$. HRMS calcd. for $C_{12}H_{21}NO_6$: 276.1447 $[M+H]^+$, found: 276.1462. ^1H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.95 (m, 1H), 2.50 (m, 1H), 2.40 (m, 2H), 3.69 (s, 3H), 3.75 (s, 3H), 4.32 (m, 1H), 5.15 (m, 1H).

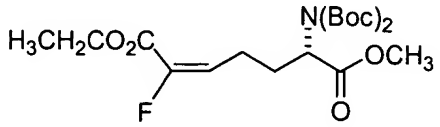


EX-A-2) To a solution of the crude product from **EX-A-1** (60 g, 0.22 mol) in 300 mL of acetonitrile at room temperature was added 4-dimethylaminopyridine (5.3 g, 0.44 mol) and di-tert-butyldicarbonate (79.2 g, 0.36 mol). The resulting mixture was stirred for 2 days at room temperature, at which time analysis by thin layer chromatography (25% ethyl acetate in hexane) showed that most of the starting material was consumed. The solvent was removed *in vacuo* affording 85 g of a red oil. The crude material was purified by flash column chromatography on silica gel eluting with 1:10 ethyl acetate in hexane to give 66.4 g (81%) of the desired di-Boc product as a pale-yellow solid. LCMS: *m/z* = 398.2 $[M+Na]^+$. HRMS calcd. for $C_{17}H_{29}NO_8$: 398.1791 $[M+Na]^+$, found: 398.1790. ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 2.19 (m, 1H), 2.41 (m, 2H), 2.46 (m, 1H), 3.66 (s, 3H), 3.70 (s, 3H), 4.91 (dd, 1H).



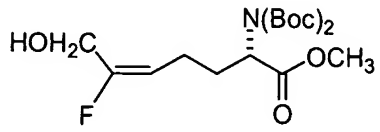
EX-A-3) A solution of DIBAL (64 mL of 1.0 M solution in hexanes, 63.9 mmol) was added dropwise to a cold solution of **EX-A-2** (20 g, 53.3 mmol) in 400 mL of anhydrous diethyl ether at -78 °C over 30 min. After an additional 30 min at -78 °C, the solution was quenched with water (12 mL, 666 mmol) and allowed to warm to room temperature. The cloudy mixture was diluted with 350 mL of

ethyl acetate, dried over $MgSO_4$ and filtered through a pad of celite. The filtrate was concentrated to a yellow oil. The crude material, 18.9 g of yellow oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 13.8 g (75%) of the desired aldehyde product as a clear oil. LCMS: *m/z* = 368.2 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 2.19 (m, 1H), 2.41 (m, 2H), 2.46 (m, 1H), 3.70 (s, 3H), 4.91 (dd, 1H), 9.8 (s, 1H).

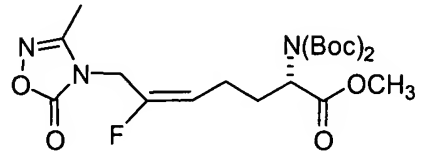


EX-A-4) To a cold (-78 °C) solution of triethyl 2-fluorophosphonoacetate (4.67 g, 19.3 mmol) in 20 mL of THF was added *n*-butyl lithium (10.9 mL of 1.6 M in hexane, 17.5 mmol). This mixture was stirred at -78 °C for 20 min producing a bright yellow solution. A solution of the product from **EX-A-3** (6.0 g, 17.5 mmol) in 5 mL of THF was then added via syringe, and the resulting mixture was stirred for 2 h at -78 °C, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The reaction was quenched at -78 °C with sat. aqueous NH_4Cl (30 mL). The organic layer was collected, and the aqueous layer was extracted with diethyl ether (2 x 50 mL). The combined organics were washed with water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concentrated. The crude material, 8.6 g of a yellow oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 6.05 g (79%) of the desired fluoro olefin product as a clear oil. ^1H NMR and ^{19}F NMR indicated that the isolated product had an approximate E:Z ratio of 95:5. LCMS: *m/z* = 456.2 $[M+Na]^+$. HRMS calcd. for $C_{20}H_{32}NO_8F$: 456.2010 $[M+Na]^+$, found: 456.2094. ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 2.0 (m, 1H), 2.25

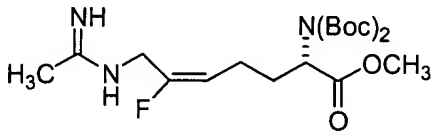
(m, 1H), 2.6 (m, 2H), 3.7 (s, 3H), 4.25 (m, 2H), 4.9 (m, 1H), 5.9 (dt, vinyl, 1H, *J* = 20 Hz), 6.2 (dt, vinyl, 1H, *J* = 30 Hz). ^{19}F NMR ($CDCl_3$) δ -129.12 (d, 0.09F, *J* = 31 Hz, 9% Z-isomer), -121.6 (d, 0.91F, *J* = 20 Hz, 91% E-isomer).



EX-A-5) To a solution of **EX-A-4** (805 mg, 1.86 mmol) in 20 mL of methanol at room temperature was added solid $NaBH_4$ (844 mg, 22.3 mmol) in 200 mg portions. The reaction was stirred for 18 h at ambient temperature, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that most of the starting material was consumed. The reaction was quenched with 20 mL of sat. aqueous NH_4Cl and extracted with ethyl acetate (2 x 35 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The crude material, 700 mg of clear oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 353 mg (48%) of the desired allylic alcohol product as a clear oil, that contained primarily the desired E-isomer by ^{19}F NMR. LCMS: *m/z* = 414.2 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 18H), 1.95 (m, 1H), 2.1 (m, 1H), 2.2 (m, 1H), 2.35 (t, 1H), 3.7 (s, 3H), 4.25 (m, 2H), 4.8 (m, 1H), 5.15 (dt, 1H, *J* = 20 Hz). ^{19}F NMR ($CDCl_3$) δ -119.1 (d, 0.02F, *J* = 37 Hz, 2% Z-isomer), -111.8 (d, 0.98F, *J* = 24 Hz, 98% E-isomer).

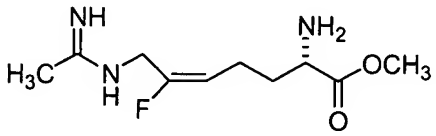


EX-A-6) To a mixture of **EX-A-5** (1.37 g, 3.5 mmol), polymer-supported triphenylphosphine (3 mmol/g, 1.86 g, 5.6 mmol) and 3-methyl-1,2,4-oxadiazolin-5-one (450 mg, 4.55 mmol) in 50 mL of THF was added dropwise dimethylazodicarboxylate (820 mg, 5.6 mmol). The reaction was stirred for 1 h at room temperature, at which time analysis by thin layer chromatography (40% ethyl acetate in hexane) showed that no starting material remained. The mixture was filtered through celite, and the filtrate was concentrated. The resulting yellow oil was partitioned between 30 mL of methylene chloride and 30 mL of water. The organic layer was separated, washed with water (1 x 30 mL) and brine (1 x 30 mL), dried over $MgSO_4$, filtered and concentrated. The crude material, 1.8 g of a yellow oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 670 mg (40%) of the desired protected E-allylic amidine product as a clear oil, that contained only the desired E-isomer by ^{19}F NMR. LCMS: *m/z* = 496.2 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 1.85 (m, 1H), 2.2 (m, 3H), 2.25 (s, 3H), 3.64 (s, 3H), 4.25 (m, 2H), 4.8 (m, 1H), 5.3 (dt, 1H, *J* = 20 Hz). ^{19}F NMR ($CDCl_3$) δ -110.8 (q, 1F, *J* = 20 Hz).



EX-A-7) The product from **EX-A-6** (670 mg, 1.4 mmol) was dissolved in 25 mL of methanol and 25 mL of 25% acetic acid in water. Zinc dust (830 mg, 12.7 mmol) was added, and the mixture was agitated under sonication for 8 h, at which time HPLC analysis showed that only 20% of the starting material remained. The Zn dust was filtered from the reaction mixture, and the filtrate was stored at -20 °C for 12 h. The filtrate was warmed to room temperature, additional glacial acetic acid (7 mL) and zinc dust (400 mg, 6.1 mmol) were

added, and the mixture was sonicated for 1 h at room temperature, at which time HPLC analysis showed 96% product. The mixture was filtered through celite, and the filtrate was concentrated. The crude material was purified by reverse-phase HPLC column chromatography on a YMC Combiprep column eluting over 8 min using a gradient of 20-95% A (A: 100% acetonitrile with 0.01% trifluoroacetic acid, B: 100% H_2O with 0.01% trifluoroacetic acid). Fractions containing product were combined and concentrated affording 344 mg (45%) of the desired acetamidine product as a trifluoroacetate salt, that contained only the desired E-isomer by ^{19}F NMR. LCMS: *m/z* = 432.3 $[M+H]^+$. ^1H NMR (CD_3OD) δ 1.52 (s, 18H), 2.9 (m, 1H), 2.2 (m, 3H), 2.27 (s, 3H), 4.2 (d, 1H), 5.4 (dt, vinyl, 1H, *J* = 20 Hz). ^{19}F NMR (CD_3OD) δ -110.83 (m, 1F, *J* = 20 Hz).

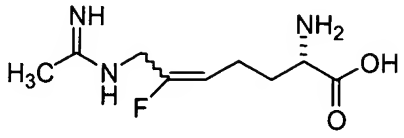


EX-A-8) A sample of the product of **EX-A-7** is dissolved in glacial acetic acid. To this stirred solution is added 10 equivalents of 1N HCl in dioxane. After stirring this solution for ten minutes at room temperature, all solvent is removed *in vacuo* to generate the illustrated methyl ester dihydrochloride salt.

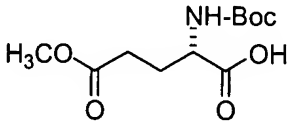
Example A) A solution of **EX-A-7** (344 mg, 1.4 mmol) in 6 mL of 6.0 N HCl was refluxed for 1 h. The solvent was removed *in vacuo*. The resulting solid was dissolved in water and concentrated three additional times, followed by 5 subsequent times in 1.0 N HCl to remove any remaining TFA salts. Upon completion, 160 mg (37%) of the desired (2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product was obtained as a white solid, m.p. 51.5-56.3 °C, that contained only the desired E-isomer by ^{19}F NMR. LCMS: *m/z* = 218.1 $[M+H]^+$. HRMS calcd. for $C_9H_{16}FN_3O_2$: 218.1305

$[M+H]^+$, found: 218.1325. ^1H NMR (D_2O) δ 1.8 (m, 2H), 2.05 (m, 2H), 2.1 (s, 3H), 3.7 (t, 1H), 4.00 (d, 2H), 5.3 (dt, vinyl, 1H, *J* = 21 Hz). ^{19}F NMR (D_2O) δ -109.9 (m, 1F, *J* = 20 Hz).

Example B

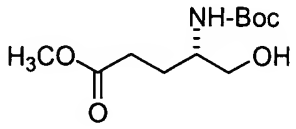


(2*S*,5*E*/*Z*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride

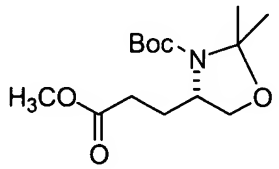


EX-B-1) To a cooled (0 °C) solution of L-glutamic acid 5-methyl ester (50.00 g, 0.31 mol) in 400 mL of 1:1 H_2O in dioxane was added triethylamine (38.35 g, 0.38 mol) followed by di-tert-butyldicarbonate (80.00 g, 0.37 mol). The resulting clear, colorless solution was allowed to stir at room temperature. After 18 h, analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The reaction mixture was quenched with 200 mL of 1.0 N aqueous $KHSO_4$. The organic layer was removed, and the aqueous layer was extracted with ethyl acetate (3 x 100 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated to give 72.00 g (89%) of the desired product as a pale yellow oil.

LCMS: *m/z* = 284.1 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.50 (s, 9H), 2.00 (m, 1H), 2.20 (m, 1H), 2.42 (m, 2H), 3.66 (s, 3H), 4.34 (d, 1H), 5.24 (d, 1H).

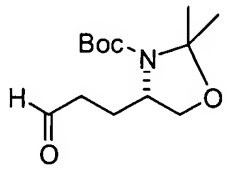


EX-B-2) To a solution of the product from **EX-B-1** (72.60 g, 0.28 mol) in 300 mL of THF at -10 °C was quickly added 4-methylmorpholine (28.11 g, 0.28 mol) and isobutylchloroformate (37.95 g, 0.28 mol). The clear yellow solution immediately formed a white precipitate. After 4 min, the resulting cloudy yellow mixture was filtered, the filtrate was cooled to -10 °C and a solution of $NaBH_4$ (15.77 g, 0.42 mol) in 200 mL of H_2O was added dropwise while maintaining a subzero temperature. Once all of the $NaBH_4$ was added, the ice bath was removed, and the reaction was allowed to stir at room temperature for 1.5 h. The reaction mixture was quenched with 200 mL of H_2O. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (3 x 100 mL). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered and concentrated to give 58 g (85%) of the desired product as a yellow oil. LCMS: *m/z* = 270.1 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.65 (m, 1H), 1.85 (m, 2H), 2.42 (t, 2H), 3.66 (s, 3H), 4.8 (d, 1H).

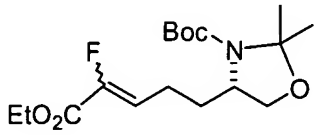


EX-B-3) To a solution of **EX-B-2** (30.95 g, 0.13 mol) in 100 mL of benzene was added 2,2-dimethoxy propane (65.00 g, 0.63 mol) followed by *p*-toluenesulfonic acid (2.40 g, 12.5 mmol) and 5 g of 3Å molecular sieves. The

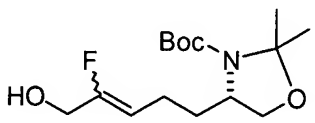
resulting mixture was refluxed for 2 h, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed complete reaction. The mixture was cooled to room temperature, diluted with diethyl ether (150 mL) and washed with sat. aqueous $NaHCO_3$ (100 mL) followed by brine (100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The crude material, 30.5 g of a yellow oil, was purified by flash column chromatography on silica gel eluting with 1:10 ethyl acetate in hexane to give 15.40 g (42%) of the desired product as a pale-yellow oil. LCMS: *m/z* = 310.1 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.42 (s, 12H), 1.56 (d, 3H), 1.85 (m, 2H), 2.38 (m, 2H), 3.66 (s, 3H), 3.7 (d, 1H), 3.95 (m, 2H).



EX-B-4) DIBAL (6.0 mL of 1.0 M solution in toluene) was added dropwise to a cold (-78 °C) solution of the product from **EX-B-3** (1.00 g, 3.00 mmol) in 10 mL of methylene chloride. After 30 min, the reaction was quenched with 5 mL sat. potassium sodium tartrate (Rochelle salt), then allowed to warm to room temperature. The mixture was then filtered through a pad of celite, dried over $MgSO_4$, re-filtered and concentrated to give a yellow oil. The crude material, 610 mg of a yellow oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 550 mg (71%) of the desired product as a clear oil. ^1H NMR ($CDCl_3$) δ 1.50 (s, 12H), 1.58 (d, 3H), 2.00 (m, 2H), 2.5 (m, 2H), 3.7 (d, 1H), 3.95 (m, 2H), 9.8 (s, 1H).

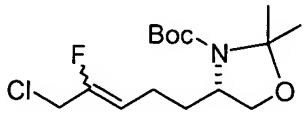


EX-B-5) To an ice cold (0 °C) solution of triethyl 2-fluoro-phosphonoacetate (6.70 g, 27.6 mmol) in 100 mL of methylene chloride was added 1,8-diazabicyclo[5.4.0]undec-7-ene (4.70 g, 31.0 mmol). The mixture was stirred at 0 °C for 1 h resulting in an orange solution. Then, a ice cold (0 °C) solution of the product from **EX-B-4** (5.71 g, 22.2 mmol) in 15 mL of methylene chloride was added via syringe, and the resulting mixture was stirred for 18 h at ambient temperature, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The solvent was removed *in vacuo*, and the resulting mixture was partitioned between 200 mL of ethyl acetate and 100 mL of water. The organic layer was collected, and the aqueous layer was extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with 1.0 M aqueous $KHSO_4$ (100 mL), water (100 mL) and brine (100 mL), dried over $MgSO_4$, filtered and concentrated to give the desired fluoro olefin product as a yellow oil (8.0 g). ^1H NMR and ^{19}F NMR indicated that the isolated product had an approximate Z:E ratio of 70:30. LCMS: *m/z* = 368.2 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 5.9-6.0 (dt, 1H, *J* = 20 Hz), 6.05-6.20 (dt, 1H, *J* = 33 Hz). ^{19}F NMR ($CDCl_3$) δ -129.89 (d, 0.7F, *J* = 38 Hz, 70% Z-isomer), -122.05 (d, 0.3F, *J* = 20 Hz, 30% E-isomer). This mixture was carried on crude without further purification.

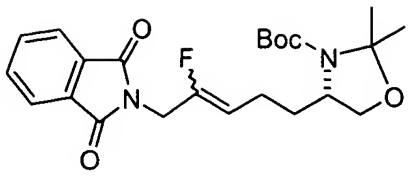


EX-B-6) To an ice cold (0 °C) solution of the product from **EX-B-5** (8.0 g, 23.0 mmol) in 70 mL of THF was added $LiBH_4$ (12.7 mL of 2.0 M in THF, 25.0 mmol) via syringe. The reaction mixture was stirred for 18 h at ambient temperature at which time analysis by thin layer chromatography (30% ethyl

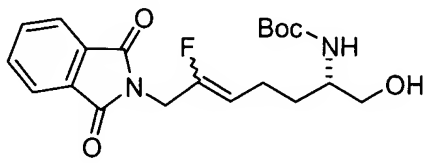
acetate in hexane) showed that no starting material remained. The THF was removed, and the resulting mixture was dissolved in methylene chloride. After cooling to 0 °C, 1.0 M aqueous $KHSO_4$ was slowly added to quench the reaction. The mixture was then extracted with ethyl acetate (3 x 50 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The crude material, 8.0 g of a clear oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 900 mg (13%) of the desired product as a clear oil. LCMS: *m/z* = 326.2 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 4.79-4.94 (dm, 1H), 5.10-5.25 (dt, 1H). ^{19}F NMR ($CDCl_3$) δ -119.82 (dt, 0.7F, *J* = 38 Hz, 70% Z-isomer), -111.09 (dt, 0.3F, *J* = 27 Hz, 30% E-isomer).



EX-B-7) To an ice cold (0 °C) solution of the product from **EX-B-6** (950 mg, 3.1 mmol) in 5 mL of pyridine was added methanesulfonyl chloride (390 mg, 3.4 mmol). The reaction was stirred for 5 min at 0 °C, then warmed to room temperature and stirred for 3 h, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The reaction was diluted with diethyl ether (10 mL) and washed with sat. aqueous $NaHCO_3$ (20 mL) followed by 1.0 M citric acid (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to give 500 mg (51%) of the desired allylic chloride product as a white solid. This product was carried forward without further purification. LCMS: *m/z* = 344.1 $[M+Na]^+$.

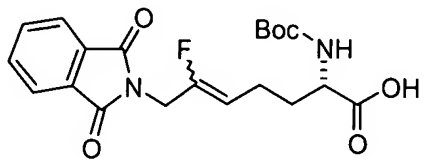


EX-B-8) To a stirring solution of the product from **EX-B-7** (440 mg, 1.37 mmol) in 10 mL of DMF was added potassium phthalimide (290 mg, 1.57 mmol). The resulting mixture was heated under reflux for 18 h, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The cooled mixture was diluted with 30 mL of water, extracted twice with ethyl acetate (30 mL), dried over $MgSO_4$, filtered and concentrated to give 540 mg (91%) of the desired product as a yellow oil. LCMS: *m/z* = 455.2 $[M+Na]^+$. HRMS calcd. for : 433.2139 $[M+H]^+$, found: 433.2144. ^1H NMR ($CDCl_3$) δ 1.4 (s, 18H), 1.6 (m, 6H), 2.05 (m, 2H), 3.6-4.42 (m, 4H), 4.9 (dt, vinyl, 1H), 5.2, (m, vinyl, 1H), 7.7 (m, 2H), 7.9 (m, 2H). ^{19}F NMR ($CDCl_3$) δ -117.09 (m, 0.7F, *J* = 38 Hz, 70% Z-isomer), -111.61 (m, 0.3F, *J* = 22 Hz, 30% E-isomer).

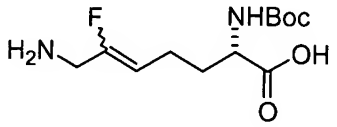


EX-B-9) The product from **EX-B-8** (600 mg, 1.38 mmol) was dissolved in 8 mL of acetic acid and 2 mL of water. The mixture was stirred at room temperature overnight at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) showed that no starting material remained. The solution was concentrated under a stream of nitrogen, and the crude product was purified by flash column chromatography on silica gel eluting with 1:2 ethyl acetate in hexane to give 248 mg (63%) of the desired product as a white

solid. LCMS: *m/z* = 415.1 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 1.56 (m, 2H), 2.15 (m, 1H), 3.64 (m, 2H), 4.35 (d, 2H), 4.9 (dt, vinyl, 1H, *J* = 37 Hz), 7.73 (m, 2H), 7.86 (m, 2H). ^{19}F NMR ($CDCl_3$) δ -116.96 (dt, 0.8F, *J* = 37 Hz, 80% Z-isomer), -111.09 (dt, 0.2F, *J* = 22 Hz, 20% E-isomer).

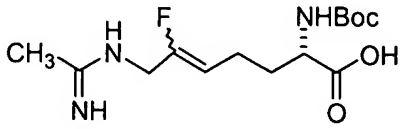


EX-B-10) To a stirring solution of the product from **EX-B-9** (237 mg, 0.605 mmol) in 6 mL of DMF was added pyridinium dichromate (1.14 g, 3.03 mmol). The solution turned dark orange and was allowed to stir at room temperature for 18 H, at which time it was poured into 20 mL of H_2O. The mixture was extracted with ethyl acetate (4 x 25 mL). The combined organic layers were washed with 5% aqueous $KHCO_3$ (3 x 25 mL). The aqueous layer was acidified with 1.0 M $KHSO_4$ to pH=3 followed by extraction with ethyl acetate (3 x 50 mL). The combined organic layers were concentrated to yield 235 mg (95%) of the desired amino acid product. The resulting white solid was carried on crude without further purification. LCMS: *m/z* = 429.1 $[M+Na]^+$.



EX-B-11) To stirring solution of the product from **EX-B-10** (230 mg, 0.56 mmol) in 7 mL of ethanol was added hydrazine hydrate (70 mg, 1.13 mmol), and the resulting solution was refluxed for 2 h forming a white precipitate. The solvent was removed *in vacuo*. The resulting white solid was dissolved in 8 mL of water and acidified to pH=4 with glacial acetic acid. It was then cooled in an ice bath and filtered. The filtrate was concentrated to give 136 mg (87%)

of the desired allyl amine product as yellow crystals which were carried onto the next step without purification. LCMS: *m/z* = 277.1 $[M+H]^+$.

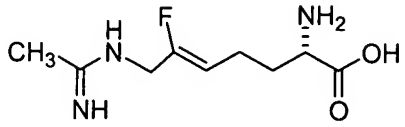


EX-B-12) To a stirring solution of the product from **EX-B-11** (136 mg, 0.50 mmol) in 6 mL of DMF was added ethyl acetimidate (252 mg, 2.04 mmol) in 3 portions over 1.5 h intervals. After the addition was complete, the mixture was stirred overnight at room temperature. The pink solution was filtered, and the filter cake was washed with water. The solvent was removed *in vacuo*, and the resulting yellow oil was purified by reverse-phase HPLC using a YMC Combiprep ODS-A semi-prep column eluting with a 7 minute gradient of 1-50% A (A: 100 acetonitrile with 0.05% TFA, B: 100 water with 0.05% TFA). Fractions containing product were combined and concentrated to afford approximately 50 mg of the desired acetamidine product as a trifluoroacetate salt which was carried onto the next step. LCMS: *m/z* = 318.2 $[M+H]^+$.

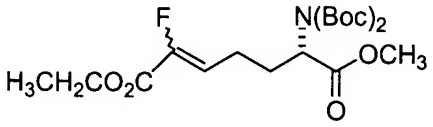
Example B) The product from **EX-B-12** was dissolved in 6 mL of 6.0 N HCl and stirred for 1 h at room temperature. The solvent was removed *in vacuo*. The resulting solid was dissolved in water and concentrated three additional times to remove TFA salts. When ^{19}F NMR indicated that all of the TFA was removed, the product was dried *in vacuo* to give 30 mg (20%, combined yield over two steps) of a 20:80 E:Z mixture containing the desired (2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride and (2*S*,5*Z*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride as a foamy clear solid. HRMS calcd. for $C_9H_{16}FN_3O_2$: 218.1305 $[M+H]^+$, found: 218.1309. ^1H NMR (D_2O) δ 2.01 (m, 2H), 2.21 (s, 3H), 2.24 (m, 2H), 3.96 (t,

1H), 4.00 (d, 2H), 5.07 (dt, vinyl, 1H, J = 37 Hz), 5.4 (dt, vinyl, 1H, J = 37 Hz). ^{19}F NMR (D_2O) δ -116.8 (m, 0.8F, J = 37 Hz, 80% Z-isomer), -109.6 (m, 0.2F, J = 21 Hz, 20% E-isomer).

Example C

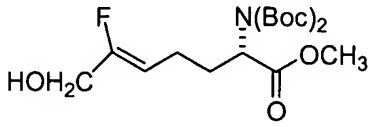


(2*S*,5*Z*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride

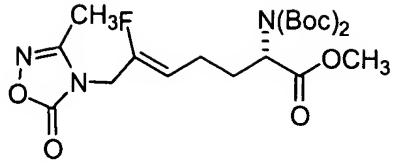


EX-C-1) Triethyl 2-fluoro-phosphonoacetate (3.54 g, 14.6 mmol) was dissolved in 20 mL of CH_2Cl_2 at 0 °C, and 1,8-diazabicyclo[5.4.0]undec-7-ene (2.4 mL, 16.4 mmol) was added. The mixture was stirred at 0 °C for 20 min producing an orange solution. A solution of the aldehyde product from **EX-A-3** (4.04 g, 11.7 mmol) was then added at 0 °C, and the resulting brown mixture was stirred overnight at room temperature, at which time LCMS indicated that no starting material remained. The solvent was removed, and the residue was partitioned between water (60 mL) and ethyl acetate (120 mL). The organic layer was collected, and the aqueous layer was extracted with ethyl acetate (2 x 50 mL). The combined organic layers were washed with water (60 mL) and 10 % aqueous $KHSO_4$ (60 mL), dried over $MgSO_4$, filtered and concentrated. The crude material, 5.7 g of an orange oil, was purified by flash column

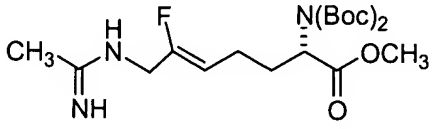
chromatography on silica gel eluting with 10% ethyl acetate in hexane to give 3.5 g (69%) of the desired fluoro olefin product as a clear oil. ^{1}H NMR and ^{19}F NMR indicated that the isolated product had an Z/E ratio of 70:30. HRMS calcd. for $C_{20}H_{32}O_8FN$: 456.2010 $[M+Na]^+$, found 456.2017. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 18H), 2.0 (m, 1H), 2.25 (m, 1H), 2.6 (m, 2H), 3.7 (s, 3H), 4.25 (m, 2H), 4.9 (m, 1H), 5.9 (dt, vinyl, 1H, *J* = 21.2 Hz), 6.1 (dt, vinyl, 1H, *J* = 32.4 Hz). ^{19}F NMR ($CDCl_3$) δ: -129.4 (d, 0.7F, *J* = 34 Hz, 70% Z isomer), -121.6 (d, 0.3F, *J* = 22 Hz, 30% E isomer).



EX-C-2) The ester product from **EX-C-1** (3.5 g, 8.1 mmol) was dissolved in 80 mL of methanol at room temperature, solid $NaBH_4$ (3 g, 80 mmol) was then added in portions. The mixture was stirred at room temperature for 18 h, at which time HPLC analysis indicated that the reaction was >90 % complete. The reaction was quenched with sat NH_4Cl. The product was extracted with ethyl acetate and dried over Na_2SO_4. The organic layer was evaporated to give 3.2 g of crude product as a colorless oil, which was purified by Biotage flash column chromatography eluting with 20% -30% ethyl acetate in hexane to give 2.11 g (67%) of a Z/E mixture of the fluoro olefin product as a clear oil along with 0.41 g (13%) of the desired pure (Z:E = 97:3 by ^{19}F NMR) Z-isomer product as a clear oil. HRMS calcd. for $C_{18}H_{30}NO_7F$: 414.1904 $[M+Na]^+$, found 414.1911. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 18H), 2.0 (m, 1H), 2.2 (m, 3H), 3.7 (s, 3H), 4.1 (dd, 2H, *J* = 17Hz), 4.8 (dt, 1H, *J* = 39 Hz), 4.9 (m, 1H). ^{19}F NMR ($CDCl_3$) δ -119.1 (dt, 1F, *J* = 39 Hz, *J* = 17 Hz).



EX-C-3) The Z-alcohol product from **EX-C-2** (390 mg, 1 mmol) and 3-methyl-1,2,4-oxadiazolin-5-one (130 mg, 1.3 mmol) were dissolved in 20 mL of THF. Then polymer supported-PPh_3 was added into the solution, and the mixture was gently stirred for 10 min. Then diethyl azodicarboxylate was added dropwise, and the mixture was stirred for 1 h at room temperature, at which time LCMS analysis indicated product formation and that no starting material was present. The polymer was filtered off through a celite pad, and the pad was washed with THF. The filtrate was evaporated to give 1.0 g of crude product which was purified by Biotage flash column chromatography eluting with 20 % to 30% ethyl acetate in hexane to give 500 mg of product, contaminated with some hydrazide by-product. This material was further purified by Biotage flash column chromatography eluting with 98:2:0.01 of methylene chloride:methanol:ammon-ium hydroxide to give 180 mg (38%) of the desired protected amidine product as a clear oil, that contained only the desired Z-isomer by ^{19}F NMR. HRMS calcd. for $C_{21}H_{32}N_3O_8F$: 491.2517 $[M+NH_4]^+$, found 491.2523. ^1H NMR ($CDCl_3$) δ 1.5 (s, 18H), 1.9 (m, 1H), 2.1 (m, 3H), 2.3 (s, 3H), 3.7 (s, 3H), 4.2 (d, 2H), 4.8 (m, 1H), 5.0 (dt, 1H, J = 36 Hz). ^{19}F NMR ($CDCl_3$) δ -116.5 (dt, 1F, J = 38 Hz).

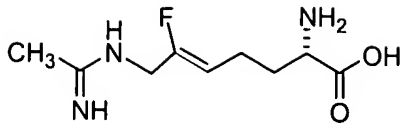


EX-C-4) The product from **EX-C-3** (88 mg, 0.19 mmol) was dissolved in 4 mL of 25% acetic acid in water containing a few drops of methanol, and then Zn

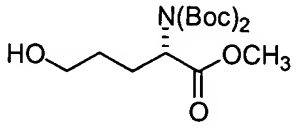
dust (109 mg, 1.67 mmol) was added. The mixture was agitated under sonication for 3 h. The Zn was filtered off through a celite pad, and the pad was washed with water. The filtrate was evaporated to dryness to give crude product which was purified by reverse-phase HPLC column chromatography on a YMC Combiprep column eluting over 8 min with a gradient of 20-80% A (A: 100% ACN with 0.01% TFA, B: 100% H_2O with 0.01% TFA). The desired product was collected in two fractions, and the combined fractions were concentrated. The product was obtained as a colorless oil as a mixture of trifluoroacetate salts that contained only the desired Z-isomer by ^{19}F NMR: 30% was mono Boc-protected product: HRMS calcd. for $C_{15}H_{26}N_3O_4F$: 332.1986 $[M+H]^+$, found 332.2001, and 70% was di-Boc-protected product: HRMS calcd. for $C_{20}H_{34}N_3O_6F$: 432.2510 $[M+H]^+$, found 432.2503. ^1H NMR of the di-Boc product (D_2O) δ 1.3 (s, 18H), 1.8 (m, 1H), 2.1 (m, 3H), 2.1 (s, 3H), 3.6 (s, 3H), 3.9 (d, 2H), 4.9 (dt, vinyl, 1H, *J* = 37 Hz). ^{19}F NMR (D_2O) δ -117.3 (dt, 1F, *J* = 37 Hz).

Example C) The combined mono- and di-BOC products from **EX-C-4** were dissolved in 30 mL of 6N HCl, and the solution was refluxed for 4 h, at which time LCMS analysis indicated complete reaction. The excess HCl and water was removed *in vacuo*. Upon completion, 9 mg (40% combined yield for two steps) of the desired (2*S*,5*Z*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product was obtained as a light yellow, very hygroscopic foam, that contained only the desired Z-isomer by ^{19}F NMR. HRMS calcd. for $C_9H_{16}N_3O_2F$: 218.1305 $[M+H]^+$, found 218.1320. ^1H NMR (D_2O) δ 1.3 (s, 18H), 1.9 (m, 2H), 2.1 (m, 2H), 2.1 (s, 3H), 3.8 (t, 1H), 3.9 (d, 2H), 4.9 (dt, vinyl, 1H, *J* = 37Hz). ^{19}F NMR (D_2O) δ -117.3 (dt, 1F, *J* = 37 Hz).

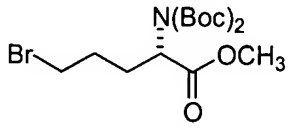
Example D



(2*S*,5*Z*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, trihydrochloride, dihydrate

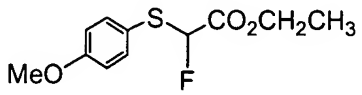


EX-D-1) The product from **EX-D-2** (3.75 g, 10 mmol) was dissolved in 60 mL of methanol, and solid $NaBH_4$ (4 g, 106 mmol) was added in portions at room temperature over 10 h, at which time HPLC analysis indicated approximately 84% reduction. The reaction mixture was quenched with sat. NH_4Cl, and was then extracted with ethyl acetate three times. The combined organic layers were dried over $MgSO_4$, filtered, and evaporated to give 3.2 g of crude product as a yellow oil. HRMS calcd. for $C_{16}H_{29}NO_7$: 348.2022 $[M+H]^+$, found: 348.2034. ^1H NMR (CD_3OD) δ 4.9 (q, 1H), 3.7 (s, 3H), 3.5 (t, 2H), 3.2 (m, 1H), 2.1 (m, 1H), 1.9 (m, 2H), 1.5 (s, 18H).

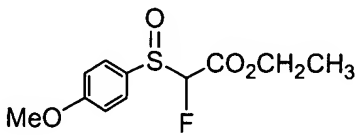


EX-D-2) The alcohol product from **EX-D-1** (3.2 g, 9.0 mmol) was dissolved in 100 mL of THF and cooled in an ice bath. Carbon tetrabromide (4.27 g, 12.9 mmol) was added, and the resulting solution was stirred at O °C for 30 min under nitrogen. Polymer-supported PPh_3 was added, and the mixture was

gently stirred at O °C for 1 h and then overnight at room temperature. The polymer was removed by filtration through celite, and the celite pad was washed with THF. The filtrate was evaporated to give crude product, which was purified by Biotage flash column chromatography eluting with 1:3 ethyl acetate in hexane to give 2.0 g (54%, combined yield over 2 steps) of the desired bromo product as a colorless oil. HRMS calcd. for $C_{16}H_{28}NO_6Br$: 410.1178 $[M+H]^+$, found: 410.1137. ^1H NMR ($CDCl_3$) δ 4.9 (q, 1H), 3.7 (s, 3H), 3.4 (m, 2H), 2.2 (m, 2H), 1.9 (m, 2H), 1.5 (s, 18H).

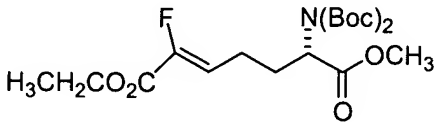


EX-D-3) A solution of NaOEt (21% in EtOH, 41.1 mL, 0.11 mol) in 60 mL of ethanol was treated with p-methoxy benzenethiol (14.0 g, 0.1 mol), followed by ethyl chlorofluoroacetate (18.3 g, 0.13 mol). The mixture was stirred at room temperature for 2 h and diluted with 250 mL of 1:1 hexane in ethyl acetate. The organic layer was washed with water three times, and dried over Na_2SO_4. The dried organic layer was evaporated to give 25 g of crude product which was carried forward without further purification. LCMS for $C_{11}H_{13}O_3SF$: *m/z* = 267.10 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 7.5 (d, 2H), 6.9 (d, 2H), 6.0 (d, 1H, *J* = 51.9 Hz), 4.2 (q, 2H), 3.8 (s, 3H), 1.2 (t, 3H). ^{19}F NMR ($CDCl_3$) δ -146.2 (d, 1F, *J* = 53.6 Hz).



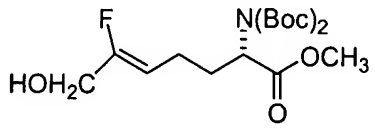
EX-D-4) A solution of the crude product from **EX-D-3** (24 g, 0.1 mol) in 200 mL of methylene chloride was cooled to -78 °C and treated with 3-

chloroperbenzoic acid (27 g, 0.12 mol) in 200 mL of methylene chloride. The reaction mixture was slowly warmed to room temperature and stirred overnight, at which time LCMS analysis indicated product formation and that no starting material remained. The solid was filtered off, and the filtrate was washed with sat. $NaHCO_3$ and NH_4Cl. The organic layer was dried over $MgSO_4$ and evaporated to give 30 g of an orange oil, which was purified by Biotage flash column chromatography eluting with 2:1 hexane in ethyl acetate to give 17.5 g (70%) of the desired sulfoxide product as an off-white oil. HRMS calcd. for $C_{11}H_{13}O_4FS$: 261.0597 $[M+H]^+$, found: 261.0598. ^1H NMR ($CDCl_3$) δ 7.6 (m, 2H), 7.0 (m, 2H), 5.6 (d, 1H, *J* = 50 Hz major diastereomer), 5.4 (d, 1H, *J* = 49 Hz minor diastereomer), 4.2 (q, 2H), 3.8 (s, 3H), 1.2 (t, 3H). ^{19}F NMR ($CDCl_3$) δ -194.3 (d, 1F, *J* = 53.6 Hz major diastereomer), -191.7 (d, 1F, *J* = 50.4 Hz minor diastereomer).

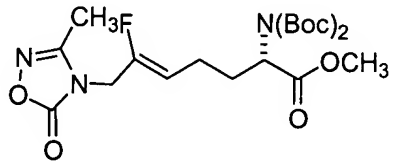


EX-D-5) A suspension of NaH (60% in mineral oil, 212 mg, 5.3 mmol) in 6 mL of dried DMF was cooled to 0 °C under nitrogen and treated with a solution of the sulfoxide product from **EX-D-4** (1.25 g, 4.8 mmol) in 2 mL of DMF. After stirring at room temperature for 20 min, the mixture was cooled to 5 °C, and the bromo product from **EX-D-2** (2.17 g, 5.3 mmol) was added in one portion. The reaction was stirred at room temperature for 3 h, then heated at reflux at 95 °C for 1 h, at which time LCMS analysis indicated product formation. The mixture was poured into an ice/aqueous NH_4Cl mixture. The product was extracted with 1:1 hexane in ethyl acetate. The organic layer was dried over Na_2SO_4 and evaporated to give 3.17 g of a crude yellow oil, which was purified by Biotage flash column chromatography eluting with 10% ethyl acetate in

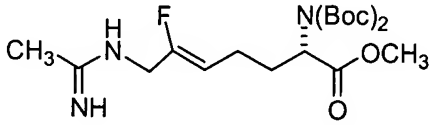
hexane to give 1.05 g (50%) of the desired fluoro olefin ester product as a colorless oil. ^{19}F NMR indicated that the isolated product contained 95:5 the desired Z-isomer. HRMS calcd. for $C_{20}H_{32}O_8FN$: 456.2010 $[M+Na]^+$, found: 456.2017. ^1H NMR ($CDCl_3$) δ 1.5 (s, 18H), 2.0 (m, 1H), 2.3 (m, 4H), 3.7 (s, 3H), 4.3 (m, 2H), 4.9 (m, 1H), 6.1 (dt, vinyl, 1H, *J* = 32.4 Hz, Z isomer). ^{19}F NMR ($CDCl_3$) δ -129.4 (d, 0.95F, *J* = 34.8 Hz, 95% Z isomer), -121.6 (d, 0.05F, *J* = 21.6 Hz, 5% E isomer).



EX-D-6) The ester product from **EX-D-5** (1.05 g, 2.4 mmol) was dissolved in methanol at room temperature, and solid $NaBH_4$ was added in portions. The mixture was stirred at room temperature for 18 h, then 2 mL of water was added, and the mixture was stirred for an additional 3 h, at which time HPLC analysis indicated the reaction was >95 % complete. The reaction was quenched with sat NH_4Cl. The product was extracted with ethyl acetate, and the organic layer was dried over Na_2SO_4 and evaporated to give 0.95 g of crude product as colorless oil. ^{19}F NMR indicated that the isolated crude product contained only the desired Z-isomer. HRMS calcd. for $C_{18}H_{30}NO_7F$: 414.1904 $[M+Na]^+$, found: 414.1949. ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 2.0 (m, 1H), 2.2 (m, 3H), 3.7 (s, 3H), 4.1 (dd, 2H, *J* = 17 Hz), 4.8 (dt, 1H, *J* = 36 Hz), 4.9 (m, 1H). ^{19}F NMR ($CDCl_3$) δ -119.1 (dt, 1F, *J* = 38 Hz, *J* = 17 Hz).



EX-D-7) The alcohol product from **EX-D-6** (0.95 g, 2.4 mmol) and 3-methyl-1,2,4-oxadiazolin-5-one (290 mg, 2.9 mmol) were dissolved in 60 mL of THF. Polymer-bound triphenyl phosphine was added, and the mixture was gently stirred for 10 min. Then dimethyl azodicarboxylate was added dropwise, and the mixture was stirred for 1 h at room temperature, at which time LCMS analysis indicated product formation and that no starting material remained. The polymer was filtered off through a celite pad, and the pad was washed with THF. The filtrate was evaporated to give a residue which was partitioned between methylene chloride and water. The organic layer was washed with water twice, dried over $MgSO_4$, and evaporated to give 1.3 g of crude product which was purified by Biotage flash column chromatography eluting with 20 % to 30% ethyl acetate in hexane to give 390 mg (34%, combined yield over 2 steps) of the desired protected amidine product as a colorless oil. ^{19}F NMR indicated that the isolated product contained only the desired Z-isomer. HRMS calcd. for $C_{21}H_{32}N_3O_8F$: 491.2517 $[M+NH_4]^+$, found: 491.2523. ^1H NMR ($CDCl_3$) δ 1.5 (s, 18H), 1.9 (m, 1H), 2.1 (m, 3H), 2.3 (s, 3H), 3.7 (s, 3H), 4.2 (d, 2H), 4.8 (m, 1H), 5.0 (dt, 1H, J = 36 Hz). ^{19}F NMR ($CDCl_3$) δ -116.5 (dt,1F, J = 38Hz).

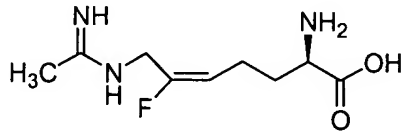


EX-D-8) The product from **EX-D-7** (390 mg, 0.82 mmol) was dissolved in 20 mL of 25% HOAc in water containing 4 mL of methanol, and Zn dust (482 mg, 7.42 mmol) was added in two portions. The mixture was agitated under sonication for 3 h. The Zn was filtered off through a celite pad, and the pad was washed with water. The filtrate was evaporated to dryness to give crude product which was purified by reverse-phase-HPLC. Fractions containing the desired products were collected, combined and concentrated. The products

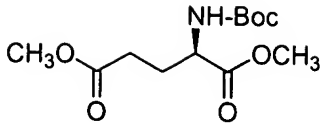
were obtained as colorless oils as a mixture of trifluoroacetate salts, that contained only the desired Z-isomer by ^{19}F NMR: 30% was mono-Boc protected product: HRMS calcd. for $C_{15}H_{26}N_3O_4F$: 332.1986 $[M+H]^+$, found 332.2001; 70% was diBoc protected product: HRMS calcd. for $C_{20}H_{34}N_3O_6F$: 432.2510 $[M+H]^+$, 432.2503. ^1H NMR of diBoc product (D_2O) δ 1.3 (s, 18H), 1.8 (m, 1H), 2.1 (m, 3H), 2.1 (s, 3H), 3.6 (s, 3H), 3.9 (d, 2H), 4.9 (dt, vinyl, 1H, *J* = 37Hz). ^{19}F NMR (D_2O) δ -117.3 (dt, 1F, *J* = 37 Hz).

Example D) The mono and diBOC products from **EX-D-8** were dissolved in 80 mL of 6N HCl and the solution was heated at reflux for 1 hour, at which time LCMS analysis indicated complete reaction. The excess HCl and water was removed *in vacuo* to give 150 mg (50% combined yield over 2 steps) of the desired (2*S*,5*Z*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, trihydrochloride, dihydrate product as a light yellow very hygroscopic foam. HRMS calcd. for $C_9H_{16}N_3O_2F$: 218.1305 $[M+H]^+$, found 218.1290. ^1H NMR (D_2O) δ 1.3 (s, 18H), 1.9 (m, 2H), 2.1 (m, 2H), 2.1 (s, 3H), 3.8 (t, 1H), 3.9 (d, 2H), 4.9 (dt, vinyl, 1H, *J* = 37 Hz). ^{19}F NMR (D_2O) δδ -117.3 (dt, 1F, *J* = 37 Hz). Anal. Calcd. for $C_9H_{16}N_3O_2F \bullet 3HCl \bullet 2H_2O$: C, 29.81; H, 6.39; N, 11.59; found C, 29.80; H, 6.11; N, 11.20.

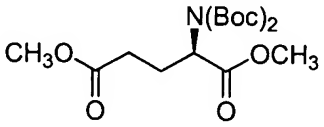
Example E



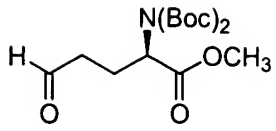
(2*R*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride, monohydrate



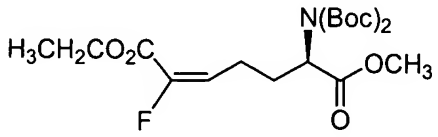
EX-E-1) Trimethylsilyl chloride is added dropwise to a cooled solution of D-glutamic acid in methanol at 0 °C. The resulting clear, colorless solution is allowed to stir at room temperature until analysis by thin layer chromatography shows that no starting material remains. The reaction is then cooled to 0 °C, triethylamine is added, and a white precipitate forms. Di-tert-butyldicarbonate is added, and the mixture is allowed to warm to room temperature. After 3 h the solvent is removed, and diethyl ether is added. The solution is filtered, and the filter cake is rinsed with additional diethyl ether. The filtrate is concentrated to give the desired mono-Boc diester product which is carried onto the next step without further purification.



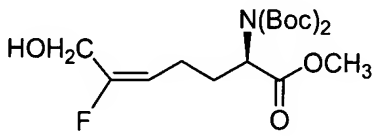
EX-E-2) To a solution of the crude product from **EX-E-1** in acetonitrile at room temperature is added 4-dimethylaminopyridine and di-tert-butyldicarbonate. The resulting mixture is stirred at room temperature, until analysis by thin layer chromatography shows that most of the starting material is consumed. The solvent is removed *in vacuo*, and the resulting residue is purified by flash column chromatography on silica gel to give the desired di-Boc protected diester product.



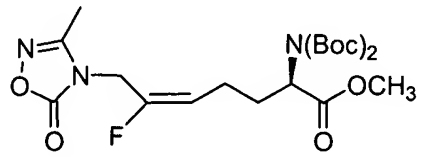
EX-E-3) A solution of DIBAL is added dropwise to a cold solution of **EX-E-2** in anhydrous diethyl ether at -78 °C. After 30 min at -78 °C, the solution is quenched with water and allowed to warm to room temperature. The resulting cloudy mixture is diluted with ethyl acetate, dried over $MgSO_4$ and filtered through a pad of celite. The filtrate is concentrated, and the resulting residue is purified by flash column chromatography on silica gel to give the desired aldehyde product



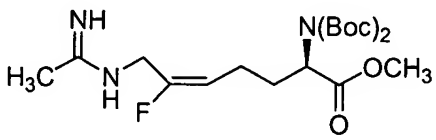
EX-E-4) To a cold (-78 °C) solution of triethyl 2-fluorophosphonoacetate in THF is added *n*-butyl lithium. This mixture is stirred at -78 °C producing a bright yellow solution. A solution of the product from **EX-E-3** in THF is then added via syringe, and the resulting mixture is stirred at -78 °C, until analysis by thin layer chromatography shows that no starting material remains. The reaction is quenched at -78 °C with sat. aqueous NH_4Cl. The organic layer is collected, and the aqueous layer is extracted with diethyl ether. The combined organics are washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The crude material is then purified by flash column chromatography on silica gel to give the desired fluoro olefin product.



EX-E-5) To a solution of **EX-E-4** in methanol at room temperature is added solid $NaBH_4$ in portions. The reaction is stirred at ambient temperature until analysis by thin layer chromatography shows that most of the starting material is consumed. The reaction is quenched with sat. aqueous NH_4Cl and extracted with ethyl acetate. The organic layers are combined, dried over $MgSO_4$, filtered and concentrated. The crude material is purified by flash column chromatography on silica gel to give the desired allylic alcohol product.



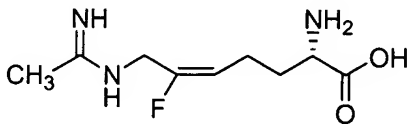
EX-E-6) To a mixture of **EX-E-5**, polymer-supported triphenylphosphine and 3-methyl-1,2,4-oxadiazolin-5-one in THF is added dropwise dimethylazodicarboxylate. The reaction mixture is stirred at room temperature until analysis by thin layer chromatography shows that no starting material remains. The mixture is filtered through celite, and the filtrate is concentrated. The resulting yellow oil is partitioned between methylene chloride and water. The organic layer is separated, washed with water and brine, dried over $MgSO_4$, filtered and concentrated. The crude material is purified by flash column chromatography on silica gel to give the desired protected E-allylic amidine product.



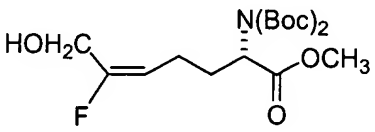
EX-E-7) The product from **EX-E-6** is dissolved in methanol and acetic acid in water. Zinc dust is added, and the mixture is agitated under sonication until HPLC analysis shows that little of the starting material remains. The Zn dust is filtered through celite from the reaction mixture, and the filtrate is concentrated. The crude material is purified by reverse-phase HPLC column chromatography. Fractions containing product are combined and concentrated affording the desired acetamidine product as a trifluoroacetate salt.

Example E) A solution of **EX-E-7** in 6.0 N HCl is refluxed for 1 h. The solvent is removed *in vacuo*. The resulting solid is dissolved in water and concentrated repeatedly from 1.0 N HCl to remove any remaining TFA salts to give the desired (2*R*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product.

Example F

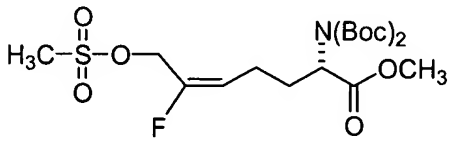


(2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride, monohydrate

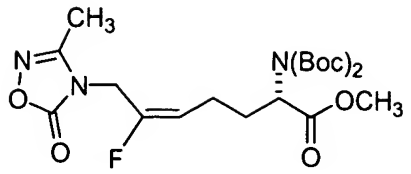


EX-F-1) To a THF (45ml) solution of the product of **EX-A-3** (5.0g, 11.5mmol) under nitrogen was added dropwise a solution of Red-Al (5.22ml, 17.4mmol) in

5.6 mL THF over 30 minutes. The internal temperature was kept below -10 °C. After 5 minutes, the reaction was quenched with 33.7ml of 1.3M Na•K tartrate. Toluene (11 mL) was added to the mixture to improve separation. The organic layer was washed with 33.7ml of 1.3M Na•K tartrate followed by brine (40 mL). The organic layers were combined, dried over MgSO4, filtered and concentrated. The crude material, 3.8 g (84%) of light yellow oil, was carried on directly into the next step. LCMS: *m/z* = 414.2 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$) δ 1.48 (s, 18H), 1.95 (m, 1H), 2.1 (m, 1H), 2.2 (m, 1H), 2.35 (t, 1H), 3.7 (s, 3H), 4.25 (m, 2H), 4.8 (m, 1H), 5.15 (dt, 1H, *J* = 20 Hz). ^{19}F NMR ($CDCl_3$) δ -119.1 (d, 0.02F, *J* = 37 Hz, 2% Z-isomer), -111.8 (d, 0.98F, *J* = 24 Hz, 98% E-isomer).

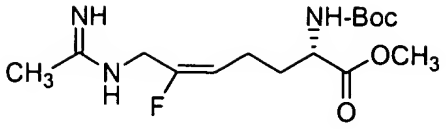


EX-F-2) To a solution of the product of **EX-F-1** (50.0 g, 0.128 mol) in 500 mL of methylene chloride at -10 °C was added triethylamine (18.0 g, 0.179 mol). A solution of methanesulfonyl chloride (17.5 g, 0.153 mol) in 50 mL methylene chloride was added slowly to maintain temperature at -10 °C. The reaction was stirred for 45 min at -10 °C, at which time analysis by thin layer chromatography (50% ethyl acetate in hexane) and LCMS showed that most of the starting material was consumed. The reaction was quenched with 600 mL of 1.0 M citric acid and extracted with ethyl acetate (2 x 400 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The crude material, 70 g of yellow oil, was carried directly into the next step. LCMS: *m/z* = 492.2 [M+Na].



EX-F-3) To a solution of the product of **EX-F-2** (70.0 g, 0.128 mol) in 400 mL of dimethyl formamide at room temperature was added potassium 3-methyl-1,2,4-oxadiazolin-5-onate (28.7 g, 0.192 mol). The reaction was stirred for 2.5 h at room temperature, at which time analysis by thin layer chromatography (30% ethyl acetate in hexane) and LCMS showed that the starting material was consumed. The reaction was diluted with 400 mL of water and extracted with ethyl acetate (5 x 400 mL). The organic layers were combined, washed with 400 mL water, 400 mL brine, dried over $MgSO_4$, filtered and concentrated. The crude material, 70 g of yellow oil, was purified by flash column chromatography on silica gel eluting with 1:4 ethyl acetate in hexane to give 38 g (63%) of a slightly yellow oil.

EX-F-4) A combination of product of several duplicate preparations of **EX-F-3** was purified by HPLC column chromatography on Merk silica gel MODCOL column at a flow of 500 mL/min isocratic at 60:40 MtBE:heptane. A second purification on the 63 g recovered was a chiral HPLC column chromatography on a Chiral Pak-AD column running at a flow of 550 mL/min isocratic at 10:90 A:B (A: 100% ethanol, B: 100% heptane). Fractions containing product were combined and concentrated affording 41 g (68%) of the desired protected L,E-allylic amidine product as a clear oil, that contained only the desired L and E-isomer by ^{19}F NMR and chiral chromatography. LCMS: *m/z* = 496.2 $[M+Na]^+$. $[M+NH_4]^+$. HRMS calcd. for $C_{21}H_{32}FN_3O_8$: 491.2507 $[M+ NH_4]^+$, found: 491.2517. ^1H NMR ($CDCl_3$) δ 1.48 (s, 18H), 1.85 (m, 1H), 2.2 (m, 3H), 2.25 (s, 3H), 3.64 (s, 3H), 4.25 (m, 2H), 4.8 (m, 1H), 5.3 (dt, 1H, *J* = 20 Hz). ^{19}F NMR ($CDCl_3$) δ -110.8 (q, 1F, *J* = 20 Hz).

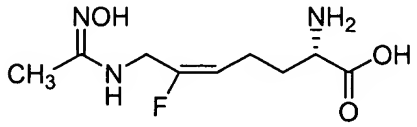


EX-F-5) The product from **EX-F-4** (22.5 g, 0.047 mol) was dissolved in 112 mL of methanol. Vigorous stirring was begun and 225 mL of 40% acetic acid in water followed by zinc dust (11.5 g, 0.177 mmol) was added. The stirring reaction was placed under reflux (approx. 60 °C) for 2.5 h, at which time HPLC analysis showed that most of the starting material had been consumed. The reaction was cooled and the Zn was filtered from the reaction mixture through celite, washing the celite well with additional methanol. The filtrate and methanol washings were combined and concentrated. The resulting oily-white solid was washed with methylene chloride (2 x 500 mL) and filtered through a celite pad, an additional 500 mL methylene chloride wash was performed. The filtrates were combined and concentrated to provide a light yellow oil. The crude material, 39 g of a light-yellow oil, was purified by plug filtration on 200 mL silica gel eluting with 80:19:1 methanol: methylene chloride: acetic acid to give 13 g (83%) of the desired product. LCMS: *m/z* = 432.3 $[M+H]^+$. 1 $[M+H]^+$. HRMS calcd. for $C_{15}H_{26}FN_3O_4$: 332.1986 $[M+H]^+$, found: 332.1982. ^{1}H NMR (CD_3OD) δ 1.42 (s, 9H), 1.7 (m, 1H), 1.9 (m, 1H), 2.17 (m, 2H), 2.22 (s, 3H), 3.3 (m, 1H), 3.7 (s, 3H), 4.2 (d, 2H), 5.1 (dt, vinyl, 1H, *J* = 21 Hz). ^{19}F NMR (CD_3OD) δ -110.83 (m, 1F, *J* = 21 Hz).

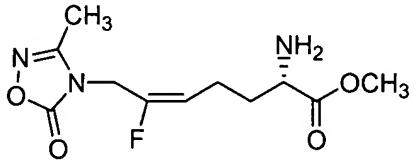
Example F) A solution of the product of **EX-F-5** (22 g, 0.066 mol) in 750 mL of 6.0 N HCl was refluxed for 45 min. The solvent was removed *in vacuo*. The resulting solid was dissolved in water and concentrated three additional times. The crude material was purified by reverse-phase HPLC column chromatography on a YMC ODS-AQ column eluting over 60 min pumping 100% isocratic B for 30 min followed by a gradient of 0-100% A for 10 min and

a 100% A wash for 20 min (A: 100% acetonitrile, B: 100% H_2O with 0.0025% acetic acid). Fractions containing product were combined and concentrated affording 3.5 g (68%) of the desired acetamidine product as a dihydorchloride salt, that contained only the desired (2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product was obtained as a white solid, m.p. 51.5-56.3 °C, that contained only the desired E-isomer by ^{19}F NMR. LCMS: *m/z* = 218.1 $[M+H]^+$. HRMS calcd. for $C_9H_{16}FN_3O_2$: 218.1305 $[M+H]^+$, found: 218.1325. ^1H NMR (D_2O) δ 1.8 (m, 2H), 2.05 (m, 2H), 2.1 (s, 3H), 3.7 (t, 1H), 4.00 (d, 2H), 5.3 (dt, vinyl, 1H, *J* = 21 Hz). ^{19}F NMR (D_2O) δ -109.9 (m, 1F, *J* = 20 Hz). $[\delta]_{589}$ = +15.3 (C, 0.334, (H_20);). $[\delta]_{365}$ = +52.8 (C, 0.334, (H_20)

Example G



(2*S*,5*E*)-2-amino-6-fluoro-7-[(1-hydroximinoethyl)amino]-5-heptenoic acid

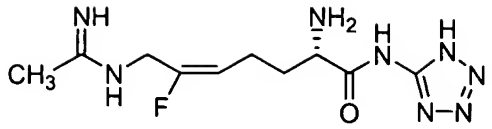


EX-G-1) Gaseous HCl was bubbled for 5 min through a stirring cold (0 °C) solution of the product of **EX-F-3** (14 g, 30.0 mmol) in 100 mL of methanol. The resulting dark yellow solution was stirred an additional 30 min, at which time HPLC indicated complete consumption of starting material. The resulting mixture was neutralized with saturated $NaHCO_3$ to pH=8, and the product was

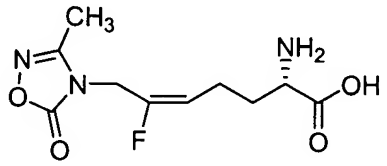
extracted out with EtOAc. The organic layer was dried over $MgSO_4$ and concentrated to give the desired amino ester product as a dark yellow oil that was carried on crude to the next step. LCMS: *m/z* = 274 $[M+Na]^+$. ^1H NMR ($CDCl_3$) δ 1.8 (m, 4H), 2.25 (s, 3H), 3.42 (bm, 1H), 3.80 (s, 3H), 4.4 (dd, 2H), 5.40 (dt, vinyl, 1H, *J* = 21 Hz). ^{19}F NMR ($CDCl_3$) δ -110.38 (m, 1F, *J* = 21 Hz).

Example G) A solution of the product of **EX-G-1** (8 g, 30 mmol) in 70 mL of 2.5N NaOH was stirred for 10 min, at which time HPLC analysis indicated the complete consumption of starting material. The resulting solution was neutralized with 12N HCl (approximately 50 mL) to pH=7-8 and concentrated. The resulting slurry was washed with methanol, filtered to remove salts and concentrated to a brownish oil. The crude material was purified by reverse-phase HPLC column chromatography on a YMC ODS-AQ column eluting over 60 min pumping 100% isocratic B for 30 min followed by a gradient of 0-100% A for 10 min and a 100% A wash for 20 min (A: 100% acetonitrile, B: 100%). Fractions containing product were combined and concentrated affording 1.0 g (14%) of the desired product as a white solid. The product was recrystallized from hot water and isopropyl alcohol and collected by filtration to afford pure (2*S*,5*E*)-2-amino-6-fluoro-7-[(1-hydroximinoethyl)amino]-5-heptenoic acid as a white crystalline solid. Melting point: 198.00-200.00 °C. LCMS: *m/z* = 234.1 $[M+H]^+$. ^1H NMR (D_2O) δ 1.8 (m, 4H), 2.05 (m, 2H), 3.6 (t, 1H), 3.9 (d, 2H), 5.2 (dt, vinyl, 1H, *J* = 21 Hz). ^{19}F NMR (D_2O) δ -112.1 (m, 1F, *J* = 20 Hz).). Anal. calcd. for $C_9H_{16}FN_3O_3$: C, 46.35; H, 6.91; N, 18.02; O, 20.58. Found: C, 46.44; H, 6.95; N, 17.94; O, 20.78. Chiral analysis >97.7%: CrownPak CR(+) at 0.8 mL/min isocratic with 100% A (A: aqueous $HClO_4$, pH=1.5).

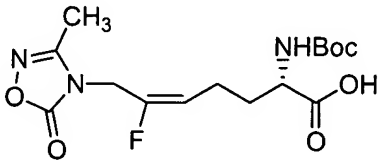
Example H



(2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]- N-(1H-tetrazol-5-yl) 5-heptenamide, dihydrochloride

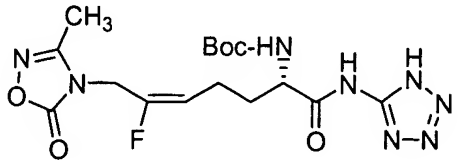


EX-H-1) The product from **EX-F-3** (6.1 g, 0.013 mol) was dissolved in 4 mL of methanol. Vigorous stirring was begun and 10 mL of 6N HCl was added. The stirring reaction was placed under reflux (approx. 60 °C) for 18 h, at which time HPLC analysis showed that most of the starting material had been consumed. The reaction was cooled and concentrated to 3.3 g (100%) of orange oil. LCMS: *m/z* = 282 $[M+Na]^+$.

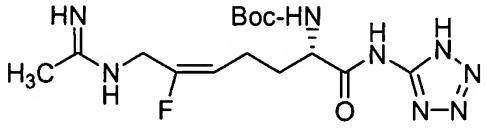


EX-H-2) The product from **EX-H-1** (3.3 g, 0.013 mol) was dissolved in 12 mL of 1:1 H_2O:dioxane. Stirring was begun and triethylamine (1.95 g, 0.019 mol) was added. The reaction was cooled to 0 °C and di-tert-butyldicarbonate (3.4 g, 0.016 mol) was added. The reaction was allowed to warm to room temperature at which time acetonitrile (4 mL) was added to dissolve solids. The reaction was stirred at room temperature for 18 h at which time HPLC analysis showed that most of the starting material had been consumed. The

reaction was quenched with 1.0N $KHSO_4$ (25 mL), extracted with ethyl acetate (3 x 50 mL) and the organic layers dried over $MgSO_4$ and concentrated. The crude material, 3.5 g of a dark oil, was purified by flash chromatography eluting with 4:95:1 methanol: methylene chloride: acetic acid to give 2.4 g (52%) of desired product as a light-yellow oil. LCMS: *m/z* = 382 $[M+Na]^+$.



EX-H-3) The product from **EX-H-2** (2.4 g, 0.007 mol) was dissolved in 13 mL THF. Stirring was begun and 5-aminotetrazole monohydrate (0.83 g, 0.008 mol) was added followed by 1,3-diisopropylcarbodiimide (1.0 g, 0.008 mol). The resulting mixture was allowed to stir at room temperature for 3 h at which time HPLC showed that most of the starting material had been consumed. To the reaction was added 12 mL water and the THF was removed by vaccum distillation. Ethanol (30 mL) was added and the reaction was heated to reflux. After 15 min at reflux, the reaction was cooled to –10 °C at which time the desired product precipitated from solution. The product was collected by filtration to afford 1.25 g (50%) of a white solid. LCMS: *m/z* = 449 $[M+Na]^+$.

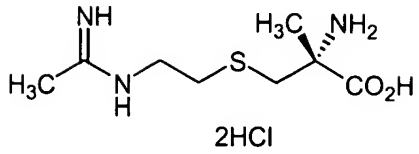


EX-H-4) The product from **EX-H-3** (1.0 g, 0.0023 mol) was dissolved in 5 mL of methanol. Vigorous stirring was begun and 10 mL of 40% acetic acid in water followed by zinc dust (0.5 g, 0.008 mol) was added. The stirring reaction was

placed under reflux (approx. 60 °C) for 1.5 h, at which time HPLC analysis showed that most of the starting material had been consumed. The reaction was cooled and the Zn was filtered from the reaction mixture through celite, washing the celite well with additional methanol. The filtrate and methanol washings were combined and concentrated. The resulting oily-white solid was purified by reverse-phase HPLC column chromatography on a YMC ODS-AQ column eluting over 60 min pumping 100% isocratic B for 30 min followed by a gradient of 0-100% A for 10 min and a 100% A wash for 20 min (A: 100% acetonitrile, B: 100% H_2O with 0.0025% acetic acid). Fractions containing product were combined and concentrated affording 0.390 g (44%) of the desired acetamidine product as a white solid. LCMS: *m/z* = 407.3 [M+Na].

Example H) The product from **EX-H-4** (0.30 g, 0.780 mmol) was dissolved in 5 mL of conc HOAc. To this was added 1 mL of 4N HCl in dioxane. The reaction was stirred 5 min. at room temperature. The solvent was removed *in vacuo*. The resulting solid was dissolved in water and concentrated three additional times. HPLC indicated amounts of starting material. The solid was dissolved in 1N HCl and stirred 3h at which time HPLC indicated that most of the starting material had been consumed. The solution was concentrated affording 290 mg (98%) of the desired acetamidine product as a dihydorchloride salt. LCMS: *m/z* = 285.1 [M+H].

Example I



***S*-[2-[(1-Iminoethyl)amino]ethyl]-2-methyl-L-cysteine, dihydrochloride**

Example-I-1) (2*R*,4*R*)-Methyl-2-tert-butyl-1,3-thiazoline-3-formyl-4-carboxylate

See Jeanguenat and Seebach, *J. Chem. Soc. Perkin Trans. 1*, 2291 (1991) and Pattenden *et al. Tetrahedron,* 49, 2131 (1993): (R)-cysteine methyl ester hydrochloride (8.58 g, 50 mmol), pivalaldehyde (8.61 g, 100 mmol), and triethylamine (5.57 g, 55mmol) were refluxed in pentane (800 ml) with continuous removal of water using a Dean-Stark trap. The mixture was filtered and evaporated. The resultant thiazolidine (9.15 g, 45 mmol) and sodium formate (3.37 g, 49.5 mmol) were stirred in formic acid (68 ml) and treated with acetic anhydride (13 mL, 138 mmol), dropwise over 1 hour at 0-5 °C. The solution was allowed to warm to RT and stir overnight. The solvents were evaporated and the residue was neutralized with aqueous 5% $NaHCO_3$ and extracted with ether (3X). The combined organic layers were dried (anhy. $MgSO_4$), filtered, and evaporated to give the title compound which was crystallized from hexane-ether as white crystals (8.65 g) (80% overall, 8:1 mixture of conformers). ^1H NMR ($CDCl_3$) δδmajor conformer: 1.04 (s, 9H), 3.29 (d, 1H), 3.31 (d, 1H), 3.78 (s, 3H), 4.75 (s, 1H), 4.90 (t, 1H), 8.36 (s, 1H). MS m/z (electrospray) 232 $(M+H)^+$ (100%), 204 (10) 164 (24).

Example-I-2) (2*R*,4*R*)-Methyl-2-tert-butyl-1,3-thiazoline-3-formyl-4-methyl-4-carboxylate

To a solution of the product of **Example-I-1**, (2*R*,4*R*)-Methyl-2-tert-butyl-1,3-thiazoline-3-formyl-4-carboxylate (8.65 g, 37.4 mmol), in anhydrous tetrahydrofuran (130 mL) under N_2 at –78 °C was added DMPU (25 mL) and the mixture stirred for 5 min. Lithium bis(trimethylsilyl)amide, 1 M in tetrahydrofuran, (37.5 mL), was added, and the mixture stirred for 30 min. After methyl iodide (5.84 g, 41.1 mmol) was added, the mixture was held at –78 °C for 4 hr and then warmed to room temperature with continuous stirring. The solvents were evaporated *in vacuo* and brine and ethyl acetate was added. The aqueous phase was extracted 3x EtOAc, and the combined organic layers were washed with 10% $KHSO_4$, water, and brine. They were then dried (anhy. $MgSO_4$), filtered, and stripped of all solvent under reduced pressure. Chromatography of the residual oil on silica with 1-10% EtOAc/hexane yielded the title compound (5.78 g, 63%, 2.4:1 mixture of conformers). ^{1}H NMR ($CDCl_3$) δδmajor conformer, 1.08 (s, 9H), 1.77 (s, 3H), 2.72 (d, 1H), 3.31 (d, 1H), 3.77 (s, 3H), 4.63 (s, 1H), 8.27 (s, 1H); minor conformer, 0.97 (s, 9H), 1.79 (s, 3H), 2.84 (d, 1H), 3.63 (d, 1H), 3.81 (s, 3H), 5.29 (s, 1H), 8.40 (s, 1H); MS m/z (electrospray) 246 $(M+H)^+$ (100%), 188 (55) 160 (95). Retention time of 16.5 min on a Daicel Chemical Industries Chiracel OAS column, 10-40% IPA/hexane 0-25 min, >95% ee.

Example-I-3) (2R) 2-Methyl-L-cysteine hydrochloride

The product of **Example-I-2**, (2*R*,4*R*)-Methyl-2-tert-butyl-1,3-thiazoline-3-formyl-4-methyl-4-carboxylate, (5.7 g, 23.2 mmol) was stirred with 6N HCl (100mL) under N_2 and held at vigorous reflux for 2 days. The solution was cooled, washed with EtOAc and evaporated to yield the product (2*R*) 2-methyl-cysteine hydrochloride (3.79 g, 95%) as a light yellow powder. ^{1}H NMR (DMSO-d_6)δδ 1.48 (s, 3H,) 2.82 (t, 1H), 2.96 (bs, 2H), 8.48 (s, 3H). MS m/z (electrospray) 136 [$M+H^+$].

Example-I-4) *S*-[2-[[(1,1-dimethylethoxy)carbonyl]amino]ethyl]-2-methyl-L-cysteine trifluoroacetate

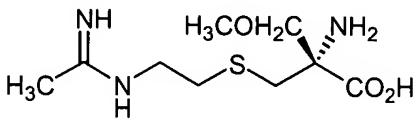
Sodium hydride (2.6 g, 60% in mineral oil, 65 mmol) was added to an oven-dried, vacuum-cooled RB flask, containing oxygen-free 1-methyl-2-pyrrolidinone (5 mL). The mixture was cooled to -10 °C and stirred under N_2. The product of **Example-I-3**, 2-Methyl-L-cysteine hydrochloride, (3.6 g, 21.0 mmol) dissolved in oxygen-free 1-methyl-2-pyrrolidinone (25 ml), was added in portions. After all H_2 evolution ceased, 2-[(1,1-dimethylethoxycarbonyl)-amino]ethyl bromide (4.94 g, 21 mmol) in oxygen-free 1-methyl-2-pyrrolidinone (15 mL) was added at -10 °C. The reaction was then stirred for 4 hr allowing warming to room temperature. The solution was neutralized with 1 N HCl and the 1-methyl-2-pyrrolidinone was removed by evaporation *in vacuo*. Reverse-phase chromatography with 1-20% acetonitrile in 0.05% aqueous trifluoro acetic acid solution yielded the title compound (5.9 g), recovered by freeze-drying appropriate fractions. ^{1}H NMR (DMSO-d_6/D_2O) δ 1.31 (s, 9H), 1.39 (s, 3H), 2.55 (m, 2H), 2.78 (d, 1H), 3.04 (d, 1H), 3.06 (t, 2H). HRMS calc. for $C_{11}H_{22}N_2O_4S$: 279.1375 ($M+H^+$), found 279.1379.

Example-I-5) S-(2-aminoethyl)-2-methyl-L-cysteine hydrochloride

The product of **Example-I-4**, *S*-[2-[[(1,1-dimethylethoxy)carbonyl]amino]ethyl]-2-methyl-L-cysteine trifluoroacetate, (5.5 g, 14.0 mmol) was dissolved in 1 N HCl (100 mL) and stirred at room temperature under nitrogen overnight. The solution was removed by freeze-drying to give the title S-(2-aminoethyl)-2-methyl-L-cysteine hydrochloride, ^{1}H NMR δ(DMSO-d_6/D_2O) δ 1.43 (s, 3H), 2.72 (m, 2H), 2.85 (d, 1 H), 2.95 (t, 2H), 3.07 (d, 1H). m/z [$M+H^+$] 179.

Example I) The product of **Example-I-5**, was dissolved in H_2O, the pH adjusted to 10 with 1 N NaOH, and ethyl acetimidate hydrochloride (1.73 g, 14.0 mmol) was added. The reaction was stirred 15-30 min, the pH was raised to 10, and this process repeated 3 times. The pH was adjusted to 3 with HCl and the solution loaded onto a washed DOWEX 50WX4-200 column. The column was washed with H_2O and 0.25 M NH_4OH, followed by 0.5 M NH_4OH. Fractions from the 0.5 M NH_4OH wash were immediately frozen, combined and freeze-dried to give an oil that was dissolved in 1N HCl and evaporated to give the title compound as a white solid (2.7 g). ^1H NMR (DMSO-d_6/D_2O) δ 1.17 (s, 3H), 2.08 (s, 3H), 2.52 (d, 1H), 2.68 (m, 2H), 2.94 (d, 1H), 3.23 (t, 2H). HRMS calc. for $C_8H_{18}N_3O_2S$: 220.1120 [M+H$^+$], found 220.1133.

Example J

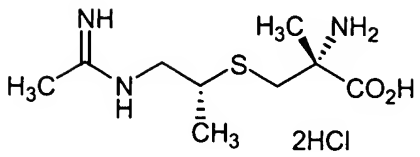


2HCl

2-[[[2-[(1-Iminoethyl)amino]ethyl]thio]methyl]-*O*-methyl-D-serine, dihydrochloride

The procedures and methods utilized in this example were identical to those of **Example I** except that in step **Example-I-2** methoxymethyl iodide was used instead of methyl iodide. These procedures yielded the title product as a white solid (2.7 g). ^1H NMR (D_2O) δ 2.06 (s, 3H), 2.70 (m, 3H), 3.05 (d, 1H), 3.23 (s, 3H), 3.32 (t, 2H), 3.46 (d, 1H), 3.62 (d, 1H). HRMS calc. for $C_9H_{20}N_3O_3S$: 250.1225 [M+H$^+$], found 250.1228.

Example K



S-[(1*R*)-2-[(1-Iminoethyl)amino]-1-methylethyl]-2-methyl-L-cysteine, dihydrochloride

Example-K-1) (*S*)-1-[(benzyloxycarbonyl)amino]-2-propanol

To a solution of (*S*)-1-amino-2-propanol (9.76 g, 130 mmol)in anhydrous benzene (60 mL) at 0 °C was added benzyl chloroformate (10.23 g, 60 mmol) in anhydrous benzene (120 mL) slowly, in portions, over a period of 20 min while vigorously stirring under an atmosphere of nitrogen. The mixture was stirred for 1 hour at 0 °C, then allowed to warm to room temperature and stirred for a further 2 hours. The mixture was washed with water (2X) and brine (2X) before the organic layer was dried over anhydrous $MgSO_4$. Evaporation of all solvent gave the title product as an oil. ^1H NMR ($CDCl_3$) δ 1.22 (d, 3H,) 2.40 (bs, 1H), 3.07 (m, 1H), 3.37 (m, 1H)), 3.94 (m, 1H), 5.16 (s, 2H), 5.27 (m, 1H), 7.38 (m, 5H). MS m/z (electrospray) 232 $[M+23]^+$(100%), 166 (96).

Example-K-2) (*S*)-1-[(benzyloxycarbonyl)amino]-2-propanol tosylate

To a solution of the product of **Example-K-1**, (*S*)-1-[(benzyloxycarbonyl)amino]-2-propanol, (9.74 g, 46.7 mmol) and triethylamine 7.27 g, 72 mmol) in methylene chloride (60 mL) at 0°C was added toluene sulfonyl chloride (9.15 g, 48 mmol) in methylene chloride (18 mL) slowly, in portions, over a period of 20 min while vigorously stirring under nitrogen. The

mixture allowed to warm to room temperature and stirred for a further 36 hours under nitrogen. The organic layer was washed with 1N HCl, water, 5% $NaHCO_3$ solution, water and brine before it was dried over anhydrous $MgSO_4$. Evaporation of all solvent gave a white solid which was passed though a silica plug with ethyl acetate/hexane (1:4) to remove excess toluene sulfonyl chloride and then with ethyl acetate/hexane (1:3) to give the title product as white crystals. This material was recrystallized from ethyl acetate/hexane to give white needles (10.8 g). ^1H NMR ($CDCl_3$) δδ1.22 (d, 3H,) 2.39 (s, 3H), 3.20 (m, 1H), 3.43 (dd, 1H)), 4.66 (m, 1H), 5.02 (m, 1H), 5.04 (ABq, 2H), 7.34 (m, 7H), 7.77 (d, 2H). MS m/z (electrospray) 386 $[M+23]^+$ (100%), 320 (66). The product was examined on a Regis Technologies Inc. Perkle Covalent (R,R) δ-GEM1 HPLC column using mobile phase of isopropanol/hexane and a gradient of 10% isopropanol for 5 min, then 10 to 40% isopropanol over a period of 25 min, and using both UV and Laser Polarimetry detectors. Retention time major peak: 22.2 min, >98 % ee.

Example-K-3) *S*-[(1*R*)-2-(Benzyloxycarbonylamino)-1-methylethyl]-2-methyl-L-cysteine trifluoroacetate

The product of **Example-I-3**, 2-methyl-L-cysteine hydrochloride, (1 g, 6.5 mmol) was added to an oven dried, N_2 flushed RB flask, dissolved in oxygen-free 1-methyl-2-pyrrolidinone (5 mL), and the system was cooled to 0 °C. Sodium hydride (0.86 g, 60% in mineral oil, 20.1 mmol) was added and the mixture was stirred at 0 °C for 15 min. A solution of the product of **Example-K-2**, (2*S*)-1-[(N-benzyloxycarbonyl)amino]-2-propanol tosylate (2.5 g, 7 mmol) dissolved in oxygen-free 1-methyl-2-pyrrolidinone (10 mL) was added over 10 min. After 15 min at 0 °C, the reaction mixture was stirred at room temperature for 4.5 hours. The solution was then acidified to pH 4 with 1N HCl and 1-methyl-2-pyrrolidinone was removed by evaporation *in vacuo*. Reverse phase chromatography with 20-40 % acetonitrile in 0.05% aqueous trifluoro acetic

acid solution yielded the title compound in (0.57g), recovered by freeze-drying. ^{1}H NMR (H_2O, 400 MHz) δ 1.0 (d, 3H), 1.4 (s, 3H), 2.6 (m, 2H), 2.8 (m, 1H), 3.1 (m, 2H), 3.6 (s, 1H), 5.0 (ABq, 2H), 7.3 (m, 5H). MS m/z (electrospray): 327 [M+H$^+$] (100%), 238 (20), 224 (10), and 100 (25).

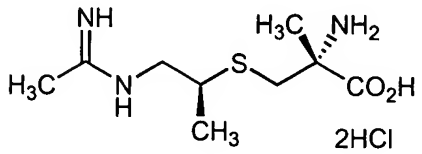
Example-K-4) *S*-[(1*R*)-2-Amino-1-methylethyl]-2-methyl-L-cysteine hydrochloride

The product of **Example-K-3**, *S*-[(1*R*)-2-(Benzyloxycarbonylamino)-1-methylethyl]-2-methyl-L-cysteine trifluoroacetate, (0.5 g, 1.14 mmol) was dissolved in 6N HCl and refluxed for 1.5 hour. The mixture was then cooled to room temperature and extracted with EtOAc. The aqueous layer was concentrated *in vacuo* to give the title product, (2*R*, 5*R*)-S- (1-amino-2-propyl)-2-methyl-cysteine hydrochloride (0.29 g), which was used without further purification. ^{1}H NMR (H_2O, 400 MHz) δ 1.2 (m, 3H), 1.4 (m, 3H), 2.7 (m, 1H), 2.8-3.2 (m, 2H), 3.4 (m, 1H). (some doubling of peaks due to rotameric forms). MS m/z (electrospray): 193 [M+H$^+$] (61%), 176 (53), 142 (34), 134 (100), and 102 (10).

Example K) The product of **Example-K-4**, *S*-[(1*R*)-2-Amino-1-methylethyl]-2-methyl-L-cysteine hydrochloride, (0.2 g, 0.76 mmol) was dissolved in 2 mL of H_2O, the pH was adjusted to 10.0 with 1N NaOH, and ethyl acetimidate hydrochloride (0.38 g, 3 mmol) was added in four portions over 10 minutes, adjusting the pH to 10.0 with 1N NaOH as necessary. After 1h, the pH was adjusted to 3 with 1N HCl. The solution was loaded onto a water-washed DOWEX 50WX4-200 column. The column was washed with H_2O and 0.5N NH_4OH. The basic fractions were pooled and concentrated to dryness *in vacuo*. The residue was acidified with 1N HCl and concentrated to the **Example K** title product, (49 mg). ^{1}H NMR (H_2O, 400 MHz) δ 1.3-1.0 (m, 3H),

1.5 (m, 3H), 2.1-1.8 (m, 3H), 3.4-2.6 (m, 5H), 3.6 (m, 1H) (rotamers observed). MS m/z (electrospray): 234 [M+H$^+$] (100%), 176 (10), and 134 (10).

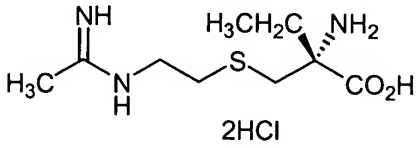
Example L



***S*-[(1*S*)-2-[(1-Iminoethyl)amino]-1-methylethyl]-2-methyl-L-cysteine, dihydrochloride**

The procedures and methods employed here were identical to those of **Example K**, except that in step **Example-K-1** (*R*)-1-amino-2-propanol was used instead of (*S*)-1-amino-2-propanol to give the title material, *S*-[(1*S*)-2-[(1-Iminoethyl)amino]-1-methylethyl]-2-methyl-L-cysteine hydrochloride. ^{1}H NMR (H_2O, 400 MHz) δ 3.6 (m, 1H), 3.4-2.6 (m, 5H), 2.1-1.8 (m, 3H), 1.5 (m, 3H), and 1.3-1.0 (m, 3H). HRMS calc for $C_9H_{19}N_3O_2S$ [M+H$^+$]: 234.1276. Found: 234.1286.

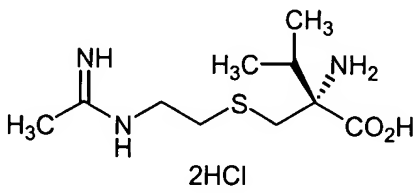
Example M



***S*-[2-[(1-Iminoethyl)amino]ethyl]-2-ethyl-L-cysteine, dihydrochloride**

The procedures and methods used in this synthesis were the same as those used in **Example I** except that ethyl triflate was used in **Example-I-2** instead of methyl iodide. Reverse phase chromatography, using a gradient of 10-40% acetonitrile in water, was used to purify the title product (20% yield). ^{1}H NMR (D_2O)δδ 0.83 (t, 3H), 1.80 (m, 2H), 2.08 (s, 3H), 2.68 (m, 1H), 2.78 (m, 1H), 2.83 (m, 1H), 3.11 (m, 1H), 3.36 (t, 2H). HRMS calc. for $C_9H_{20}N_3O_2S$: 234.1276 [M+H$^+$], found 234.1284.

Example N



2-[[[[2-(1-Iminoethyl)amino]ethyl]thio]methyl]-D-valine, dihydrochloride

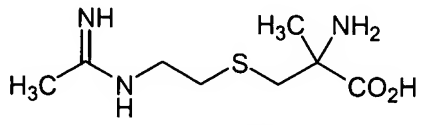
Example-N-1) Isopropyl triflate

Silver triflate (25.25 g, 98.3 mmol) stirred in diethyl ether (300 mL) under nitrogen was treated with isopropyl iodide (16.54 g, 98.5 mmol) in ether (200 mL) over 15 minutes. The mixture was stirred for 10 minutes and then filtered. The filtrate was distilled at reduced pressure. The distillate was redistilled at atmospheric pressure to remove the majority of the diethyl ether, leaving a mixture of the title isopropyl triflate-diethyl ether (84:16 by weight) (15.64 g, 70% corrected) as a colorless liquid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.52 (d, 6H), 5.21 (septet, 1H).

The procedures and methods utilized here were the same as those used in **Example I** except that isopropyl triflate replaced methyl iodide in **Example-I-2**.

The crude title product was purified by reversed phase chromatography using a gradient elution of 10-40% acetonitrile in water. ^{1}H NMR (H_2O, 400 MHz) δδ 0.94 (dd, 6H), 2.04 (septet, 1H), 2.10 (s, 3H), 2.65, 2.80 (d m, 2H), 2.85, 3.10 (dd, 2H), 3.37 (t, 2H). HRMS calc. for $C_{10}H_{22}N_3O_2S$: 248.1433 [M+H$^+$], found 248.1450.

Example O



2TFA

***S*-[2-(1-Iminoethylamino)ethyl]-2-methyl-(D/L)-cysteine, bistrifluoroacetate**

Example-O-1) *S*-(2-aminoethyl)-L-cysteine, methyl ester

A 10 g (50 mmol) sample of *S*-(2-aminoethyl)-L-cysteine was dissolved in 400 mL of methanol. Into this cooled solution was bubbled in anhydrous HCl for 30 minutes. After stirring at room temperature overnight, the solution was concentrated to afford 12.7 g of the title compound.

Example-O-2) *N*-{4-chlorophenyl)methylene]-*S*-[2-[[(4-chlorophenyl)methylene]amino]ethyl]-L-cysteine, methyl ester

A 12.7 g (50 mmol) sample of the product of **Example-O-1**, *S*-(2-aminoethyl)-L-cysteine methyl ester, was dissolved in acetonitrile. To this solution was added 12.2 g (100 mmol) of anhydrous $MgSO_4$, 14g (100 mmol) of 4-chlorobenzaldehyde and 100 mmol of triethylamine. This mixture was stirred for 12 hours, concentrated to a small volume and diluted with 500 mL of ethyl acetate. The organic solution was washed successively with (0.1%) $NaHCO_3$, (2N) NaOH, and brine solution. The organic was dried (anhy.

$MgSO_4$), filtered and concentrated to afford 7.5g of the title compound. [M + H^+] = 179.

Example-O-3) *N*-[4-chlorophenyl)methylene]-*S*-[2-[[(4-chlorophenyl)methylene]amino]ethyl]-2-methyl-D/L-cysteine methyl ester

A sample of the product of **Example-O-2**, *N*-{4-chlorophenyl)methylene]-*S*-[2-[[(4-chlorophenyl)methylene]amino]ethyl]-L-cysteine methyl ester (7.5 g, 17 mmol), in anhydrous THF was treated with 17 mmol of sodium bis(trimethylsilyl)amide at -78 °C under nitrogen, followed by 2.4g (17mmol) of methyl iodide. The solution was held at -78 °C for 4 hr and then warmed to room temperature with continuous stirring. The solvents were evaporated *in vacuo* and brine and ethyl acetate was added. The aqueous phase was extracted 3x EtOAc, and the combined organic layers were washed with 10% $KHSO_4$, water, and brine before it was dried (anhy. $MgSO_4$), filtered, and evaporated to afford the title compound.

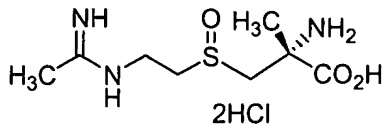
Example-O-4) *S*-(2-aminoethyl)-2-methyl-D/L-cysteine, hydrochloride

A sample of the product of **Example-O-3**, *N*-[4-chlorophenyl)methylene]-*S*-[2-[[(4-chlorophenyl)methylene]amino]ethyl]-2-methyl-D/L-cysteine methyl ester (4.37 g, 10 mmol), was stirred and heated (60 °C) with 2N HCl overnight and the solution washed (3X) with ethyl acetate. The aqueous solution was freeze-dried to give the title compound.

Example O) A sample of the product of **Example-O-4**, *S*-(2-aminoethyl)-2-methyl-D/L-cysteine dihydrochloride (2.5 g (10 mmol), was dissolved in H_2O and the pH was adjusted to 10 with 1 N NaOH. Ethyl acetimidate hydrochloride (1.24 g, 10.0 mmol) was then added to the reaction mixture. The reaction was stirred 15-30 min, the pH was raised to 10, and this process

repeated 3 times. The pH was reduced to 4 with HCl solution and the solution evaporated. The residue was purified on reverse phase HPLC with H_2O containing 0.05% trifluoroacetic acid as the mobile phase to afford the **Example O** title product. M + H = 220.

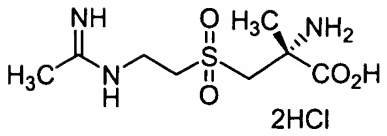
Example P



(2*R*)-2-Amino-3[[2-[(1-iminoethyl)amino]ethyl]sulfinyl]-2-methylpropanoic acid, dihydrochloride

A solution of *S*-[2-[(1-iminoethyl)amino]ethyl]-2-methyl-L-cysteine, dihydrochloride (**Example I**, 0.2g, 0.73 mmol) in 3 mL of water was stirred and cooled to 0 °C and a solution of 3% H_2O_2 (0.8 mL, 0.73 mmol) in formic acid (0.4 mL, 0.73 mmol) was added in 0.3 mL portions. The cold bath was removed and the reaction mixture was stirred at room temperature for 48 hours. The solution was concentrated *in vacuo*, diluted with of water (10 mL) and concentrated again to give the crude sulfone. This residue was chromatographed (C-18 reverse phase, with mobile phase H_2O containing 0.05% trifluoroacetic acid) to give the pure sulfone. The sulfone was treated with 1M HCl (10 mL) and concentrated *in vacuo* to give 140 mg of a mixture of 2 diastereomers of the title compound as a colorless oil of the HCl salts. ^1H NMR (300 MHz, D_2O) δ 1.5 (s, 2H), 1.6 (s, 1H), 2.0 (s, 3H), 3.1 (m, 2H), 3.3 (m, 2H) 3.6 (m, 2H). HRMS calc. for $C_8H_{18}N_3O_3S$: 236.1069 [M+H$^+$], found: 236.1024.

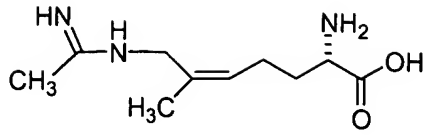
Example Q



(2*R*)-2-Amino-3[[2-[(1-iminoethyl)amino]ethyl]sulfonyl]-2-methylpropanoic acid dihydrochloride

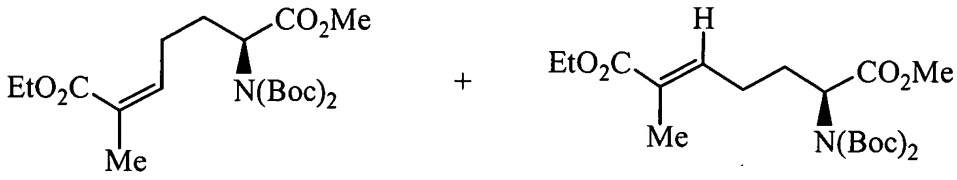
A solution of *S*-[2-[(1-Iminoethyl)amino]ethyl]-2-methyl-L-cysteine dihydrochloride, the product of **Example I**, (0.15 g, 0.54 mmol) in 2 mL of water was cooled to 0 °C and a solution of 3% H_2O_2 (1.6 mL, 1.46 mmol) in formic acid (0.8mL, 14.6 mmol) was added. The cold bath was removed and the reaction mixture was stirred at room temperature for 18 hours. The solution was concentrated *in vacuo*, diluted with 10 mL of water and concentrated again to give the crude sulfoxide. The residue was diluted with 4 mL of water and was adjusted to pH 9 with 2.5 N NaOH. Acetone (5 mL) was added, followed by Boc_2O (0.2 g), and the reaction was stirred for 48 h at room temperature. The reaction mixture was adjusted to pH 6 with 1M HCl and was concentrated *in vacuo*. This residue was chromatographed (C-18 reverse phase; 40 to 50% ACN: H_2O, 0.05% TFA) to give the pure Boc protected material. The fractions were concentrated *in vacuo* and the residue was treated with 1N HCl (3 mL) for 1h. The solution was concentrated to give 30 mg of the title compound as colorless oil. ^{1}H NMR (400 MHz, D_2O) δ 4.0 (d, 1H), 3.7 (d, 1H), 3.6 (t, 2H), 3.5 (t, 2H), 2.1 (s, 3H), and 1.5 (s, 3H) ppm. HRMS calc. for $C_8H_{18}N_3O_4S$: 252.1018 [M + H^+], found: 252. 0992.

Example R



(2*S*,5*Z*)-2-amino-6-methyl-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride

Example R-1)

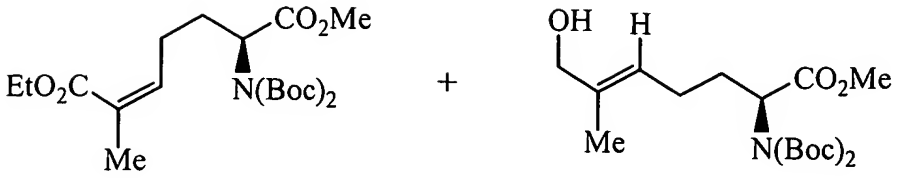


Z E

A solution of triethyl-2-phosphonopropionate (6.5 mg, 27.1 mmol) in toluene (60 ML) was treated with 0.5 M potassium bis(trimethylsilyl) amide (50.0 ML, in toluene) and the resulting anion was condensed with the aldehyde product of **Example U-3** by the method of **Example U-4** (*see* **Example U** *infra*). This produced, after chromatography, 8 g of a 3:7 mixture respectively of the desired Z and E diesters.

(^{1}H)NMR (300 MHz, CDCl3) 6.7-6.8 ppm (m,1H), 5.9 ppm (m,1H), 4.9 ppm (m, 1H), 4.2 ppm (q, 2H), 3.7 ppm (s, 3H), 2.5 ppm (m, 1H), 2.2-2.3 ppm (m, 2H), 2.0 ppm (m, 1H), 1.9 ppm (s, 3H), 1.8 ppm (s, 3H), 1.5 ppm (s, 18H), 1.3 ppm (t, 3H).

Example R-2)

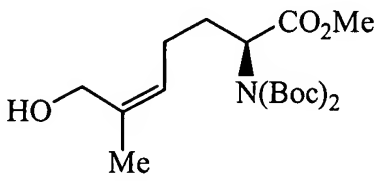


Z E

The product mixture of **Example R-1** (850 mg, 2.0 mmol) in Et_2O (30 mL) was reduced over a period of twenty minutes with diisobutyl aluminum/hydride (DIBAL) by the method of **Example U-5** to produce the crude illustrated desired mixture of E-alcohol and unreduced Z-ester. This mixture was chromatographed on silica gel eluting with n-hexane : EtOAc (9:1) to n-hexane : EtOAc (1:1) providing samples of the Z-ester (530 mg) and the E-alcohol desired materials.

Z- ester: (^{1}H)NMR (300 MHz, CDCl3) 5.9 ppm (m,1H), 4.9 ppm (m, 1H), 4.2 ppm (q, 2H), 3.7 ppm (s, 3H), 2.5 ppm (m, 1H), 2.2-2.3 ppm (m, 2H), 1.9 ppm (s, 3H), 1.5 ppm (s, 18H), 1.3 ppm (t, 3H).
E- alcohol: (^{1}H)NMR (300 MHz, CDCl3) 5.35 ppm (m,1H), 4.9 ppm (m, 1H), 3.95 ppm (s, 1H), 3.7 ppm (s, 3H), 1.8-2.2 ppm (m, 6H), 1.6 ppm (s, 3H), 1.5 ppm (s, 18H).

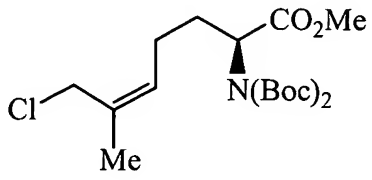
Example R-3)



The product Z-ester of **Example R-2** (510 mg, 1.2 mmol) in Et_2O (30 ML) was reduced over a period of two hours with diisobutyl aluminum/hydride (DIBAL) by the method of **Example U-5** to produce the crude illustrated desired Z-alcohol. This material was chromatographed on silica gel eluting with n-hexane : EtOAc (9:1) to n-hexane : EtOAc (8:2) to yield 340 mg of the desired Z-alcohol product.

(^{1}H)NMR (300 MHz, $CDCl_3$) δ 5.3 ppm (m,1H), 4.9 ppm (m, 1H), 4.2 ppm (d, 1H), 4.0 ppm (d, 1H), 2.2 ppm (m, 3H), 1.95 ppm (m, 1H), 1.8 ppm (s, 3H), 1.5 ppm (s, 18H).

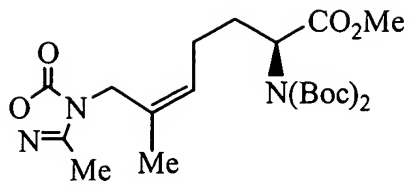
Example R-4)



A CH_2Cl_2 solution (5 ML) of the product alcohol of **Example R-3** (340 mg, 0.9 mmol) was treated with triethylamine (151 mg, 1.5 mmol). To this solution cooled in an ice bath was added a CH_2Cl_2 solution (1.5 ML) of methanesulfonyl chloride. After fifteen minutes the ice bath was removed and the reaction was stirred at ambient temperature for 20 h. The reaction mixture was then washed with 10% $KHSO_4$, dried over Na_2SO_4, and stripped of all solvent under reduced pressure to produce 350 mg of the desired Z-allylic chloride.

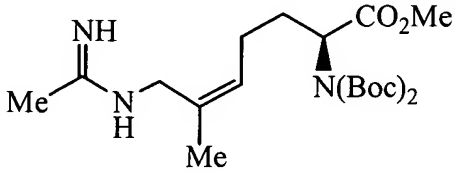
(^{1}H)NMR (300 MHz, $CDCl_3$) δ 5.4 ppm (m,1H), 4.9 ppm (m, 1H), 4.1 ppm (d, 1H), 4.0 ppm (d, 1H), 2.1 ppm (m, 3H), 1.95 ppm (m, 1H), 1.8 ppm (s, 3H), 1.5 ppm (s, 18H).

Example R-5)



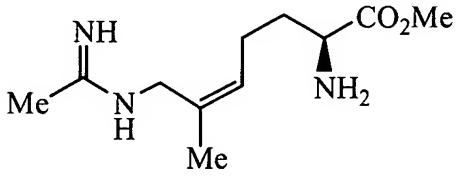
A suspension of potassium 3-methyl-1,2,4-oxa-diazoline-5-one in DMF is reacted with a DMF solution of the product of **Example R-4** by the method of **Example S-2** *infra* to produce the material.

Example R-6)



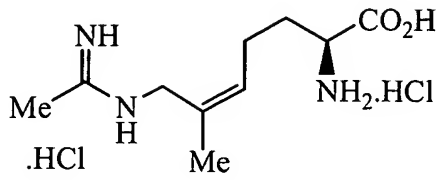
The product of **Example R-5** is reacted with zinc in HOAc by the method of **Example U-7** to yield the amidine.

Example R-7)



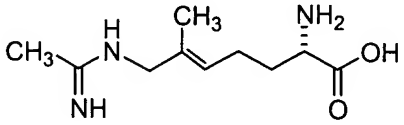
The product of **Example R-6** was reacted with 4NHCl in dioxane in glacial HOAc to yield the amidine.

Example R)



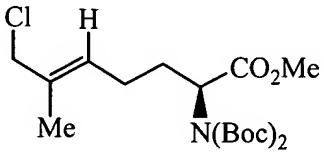
The product of **Example R-7** is deprotected to yield the amino acid, dihydrochloride.

Example S



(2*S*,5*E*)-2-amino-6-methyl-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride

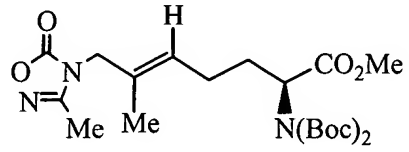
Example S-1)



The E-alcohol product of **Example R-2** (1.3 g, 3.3 mmol) was reacted with triethylamine (525 mg, 5.2 mmol) and methanesulfonyl chloride (560 mg, 5.2 mmol) by the method of **Example R-4** to yield 1.4 g of the desired E-allylic chloride.

(^{1}H)NMR (400 MHz, CDCl3) 5.5 ppm (m,1H), 4.9 ppm (m, 1H), 4.0 ppm (s, 2H), 3.7 ppm (s, 3H), 2.1-2.3 ppm (m, 3H), 1.9 ppm (m, 1H), 1.7 ppm (s, 3H), 1.5 ppm (s, 18H).

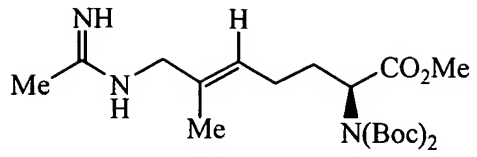
Example S-2)



A suspension of potassium 3-methyl-1,2,4-oxa-diazoline-5-one (460 mg, 3.35 mmol) in 5 mL of DMF was treated with a DMF (15 mL) solution of the product of **Example S-1**. This reaction mixture was stirred at 50 °C for 17 h before an additional 50 mg (0.04 mmol) of the diazoline-5-one salt was added. Heating of the stirred reaction was continued for an additional 3 h before it was cooled to room temperature and diluted with 180 mL of water. This mixture was extracted with EtOAc and the extracts were diluted with 120 mL of n-hexane, washed with water, dried over Na_2SO_4 and stripped of all solvent under reduced pressure to yield 1.3 g of the material.

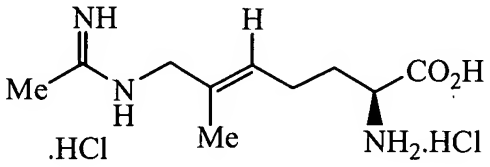
(^{1}H)NMR (400 MHz, CDCl3) 5.5 ppm (m,1H), 4.9 ppm (m, 1H), 4.2 ppm (s, 3H),3.7 ppm (s, 3H), 2.2 ppm (m, 3H), 1.95 ppm (m, 1H), 1.8 ppm (s, 3H), 1.5 ppm (s, 18H).

Example S-3)



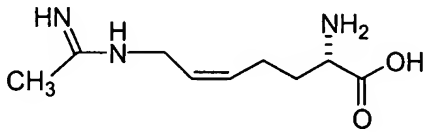
The product of **Example S-2** (460 mg, 1.0 mmol) was reacted with zinc in HOAc by the method of **Example U-7** (*see* **Example U** *infra*) to yield 312 mg of the desired amidine after HPLC purification.

Example S)

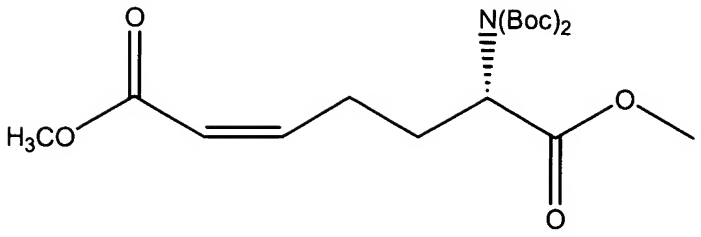


The product of **Example S-3** (77 mg, 0.2 mmol) was deprotected with 2N HCl by the method of **Example U** to yield 63 mg the E-amino acid, dihydrochloride.

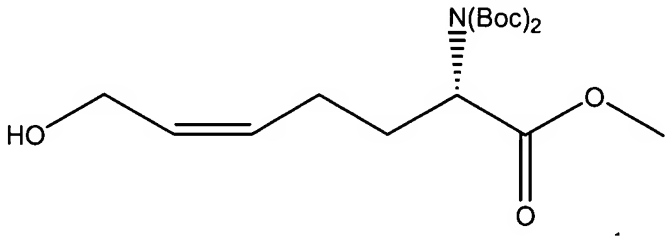
Example T



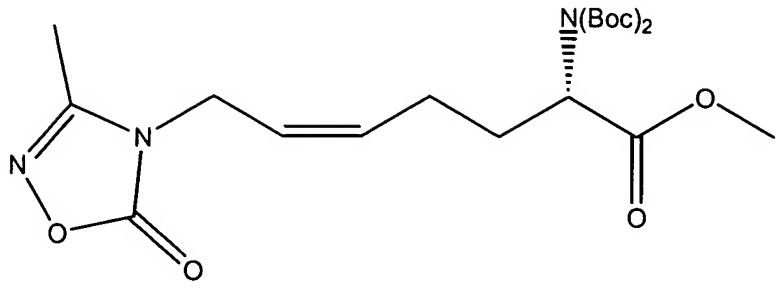
(2*S*,5*Z*)-2-amino-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride



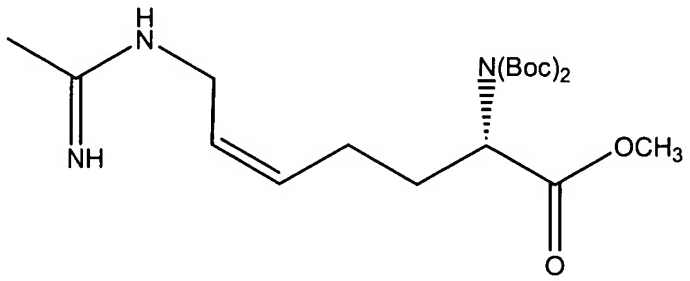
Example T-1) Methyl bis(trifluoroethyl)phosphonoacetate (4.77 g, 15 mmol) and 23.7g (90 mmol) of 18-crown-6 were dissolved in 80 mL of anhydrous THF and cooled to -78 ° C. To this soution was added 30 mL (15 mmol) of potassium bis(trimethylsilyl) amide, followed by 5.1g (14.7 mmol) of N,N-diBoc glutamic aldehyde methyl ester from **Example U-3** (*see* **Example U** *infra*). After stirring for 30 minutes at -78 ° C, the reacion was quenched with aqueous $KHSO_4$. Extraction of the reaction mixture with EtOAc and concentration afforded 2.95g (49%) of the desired compound. Mass spectra M + H = 402.



Example T-2) The product from **Example T-1** was reduced by the method of **Example U-5** to afford the desired compound.



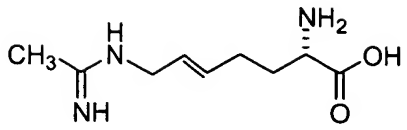
Example T-3) The product from **Example T-2** was allowed to react with 3-methyl-1,2,4-oxadiazolin-5-one by the method of **Example U-6** to afford the desired compound.



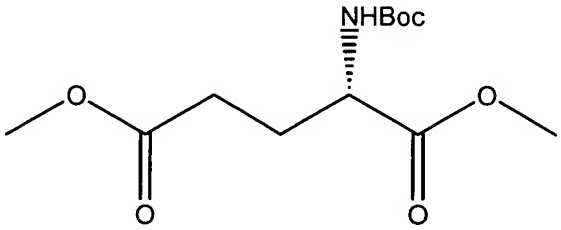
Example T-4) The product from **Example T-3** was deprotected by the method of **Example U-7** to afford the desired compound.

Example T) The product from **Example T-4** was dissolved in 2 N HCl and heated at reflux. The reaction mixture was cooled and concentrated to afford 0.12 g of the desired product. H^1- NMR 1.8-2.0 (m, 2H); 2.05 (s, 3H); 2.15 (q, 2H); 3.75 (d, 2H); 3.9 (t, 1H); 5.45 (m, 1H); 5.6 (m, 1H)

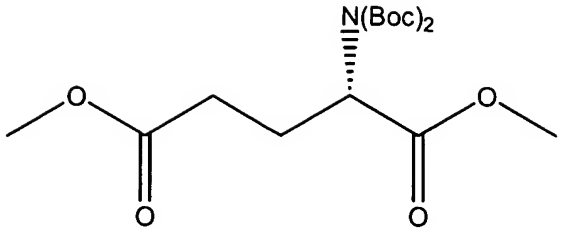
Example U



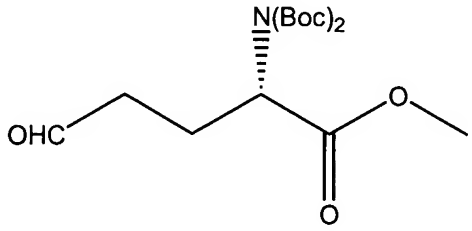
(2*S*,5*E*)-2-amino-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride



Example U-1) L-glutamic acid (6.0g, 40.78 mmol) was dissolved in methanol (100 mL). To the reaction mixture trimethylsilyl chloride (22.9 mL, 180 mmol) was added at 0 °C under nitrogen and allowed to stir overnight. To the reaction mixture at 0 ° C under nitrogen triethylamine (37 mL, 256 mmol) and di-tert-butyldicarbonate (9.8 g, 44.9 mmol) was added and stirred two hours. The solvent was removed and the residue was triturated with ether (200 mL). The triturated mixture was filtered. The filtrate was evaporated to an oil and chromatographed on silica, eluting with ethyl acetate and hexane, to give the mono boc L-glutamic diester (10.99 g, 98%).



Example U-2) Mono boc L-glutamic acid (10.95 g, 39.8 mmol) was dissolved in acetonitrile (130 mL). To the reaction mixture 4-dimethylaminopyridine (450 mg, 3.68 mmol) and di-tert-butyldicarbonate (14.45 g, 66.2 mmol) was added and stirred for 20 hours. The solvent was evaporated and the residue chromatographed on silica and eluting with ethyl acetate and hexane to give the di-boc-L-glutamic diester (14.63 g, 98 %).

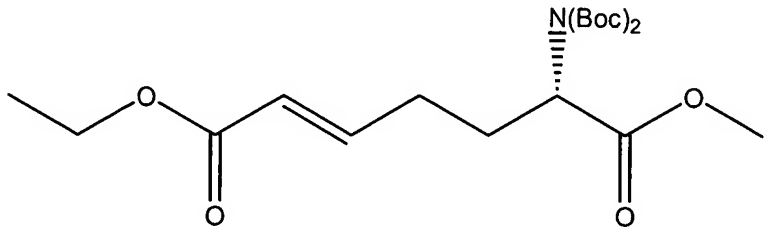


Example U-3) The product from **Example U-2** (10.79 g, 28.7 mmol) was dissolved in diethyl ether (200 mL) and cooled in a dry ice bath to -80 C. To the reaction mixture Diisobutylaluminum hydride (32.0 mL, 32.0 mmol) was added and stirred 25 minutes. The reaction mixture was removed from the dry ice bath and water (7.0 mL) was added. Ethyl acetate (200 mL) was added to the reaction mixture and stirred 20 minutes. Magnesium sulfate (10g) was added to the reaction mixture and stirred 10 minutes. The reaction mixture was filtered through celite and concentrated to give a clear yellow oil (11.19g).

The yellow oil was chromatographed on silica and eluting with ethyl acetate and hexane. The product (8.61, 87 %) was a clear light yellow oil.

Mass Spectrometry: M+H 346, M+Na 378

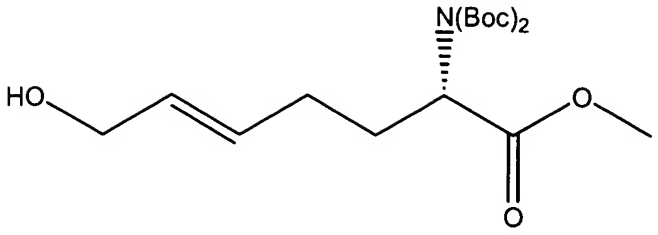
(^{1}H)NMR (400 MHz, $CDCl_3$) 9.74 ppm (s, 1H), 4.85 ppm (m, 1H), 3.69 ppm (s, 3H), 2.49 ppm (m, 3H), 2.08 ppm (m, 1H), 1.48 ppm (s, 18H).



Example U-4) Triethyl phosphonoacetate (6.2 mL, 31.2 mmol) was dissolved in toluene (30 mL) and placed in an ice bath under nitrogen and cooled to 0 $^{\circ}$ C. To the reaction mixture, potassium bis(trimethylsilyl) amide (70 mL, 34.9 mmol) was added and stirred 90 minutes. To the reaction mixture the product from **Example U-3** (8.51 g, 24.6 mmol) dissolved in toluene (20 mL) was added and stirred 1 hour. The reaction mixture was warmed to room temperature. To the reaction mixture Potassium hydrogen sulfate (25 mL, 25 mmol) was added and stirred 20 minutes. The mixture was extracted with ethyl acetate (3x100 mL), dried over Magnesium sulfate and concentrated to give a cloudy brownish yellow oil (12.11 g). The oil was chromatographed on silica, eluted with ethyl acetate and toluene to give a light yellow oil (7.21 g, 70 %).

Mass Spectrometry: M+H 416, M+NH_4 433, -boc 316, -2 boc, 216.

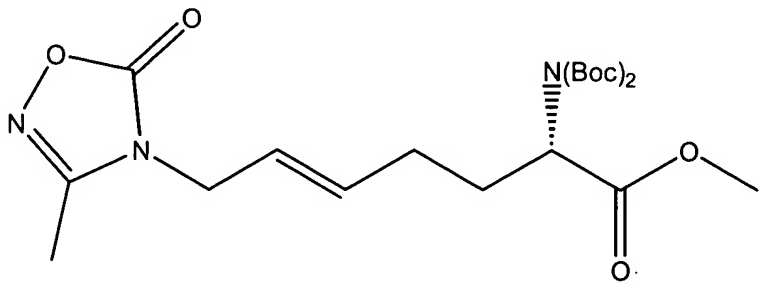
(^{1}H)NMR (400 MHz, $CDCl_3$) 6.88 ppm (m, 1H), 5.82 ppm (d, 1H), 4.81 ppm (m, 1H), 5.76 ppm (s, 3H), 2.50ppm (m, 3H), 2.21 ppm (m, 1H), 1.45 ppm (s, 18H).



Example U-5) The product from **Example U-4** (5.0 g, 12.03 mmol) was dissolved in diethyl ether (100 mL) and placed in a dry ice bath and cooled to -80 •C. To the reaction mixture was added diisobutylaluminum hydride (21.0 mL, 21.0 mmol). And stirred 30 minutes. To the reaction mixture water (10 mL) was added, removed from dry ice bath, and stirred 60 minutes. To the reaction mixture magnesium sulfate (10 g) was added and stirred 10 minutes. The reaction mixture was filtered over celite and concentrated to give a yellow oil (5.0 g). The oil was chromatographed on silica, eluted with ethyl acetate and hexane, to give a light yellow oil (2.14 g, 47 %).

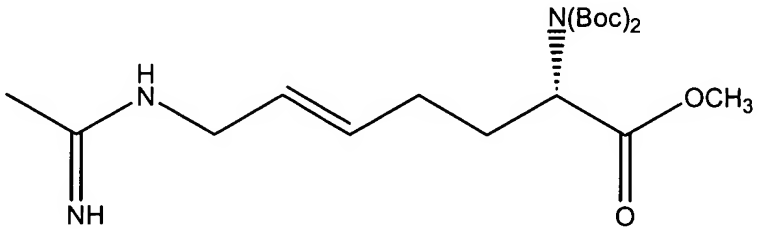
Mass Spectrometry: M+H 374, M+NH_4 391

(^{1}H)NMR (400 MHz, $CDCl_3$) 5.63 ppm (m, 2H), 4.88 ppm (m, 1H), 4.02 ppm (s, 2H), 3.68 ppm (s, 3H), 2.12 ppm (m, 4H), 1.47 ppm (s, 18H).



Example U-6) The product from **Example U-5** was dissolved in tetrahydrofuran (50mL). To the reaction mixture triphenyl phosphine on polymer (3.00 g, 8.84 mmol), oxadiazolinone (720 mg, 7.23 mmol), and azodicarboxylic acid dimethyl ester (1.17 g, 3.21 mmol) were added and stirred six hours at room temperature. The reaction mixture was filtered over celite and concentrated to give a cloudy yellow oil (2.81 g). The oil was chromatographed on silica, eluting with ethyl acetate in hexane, to give a clear colorless oil (1.66 g, 68 %).

Mass Spectrometry: M+H 456, M+NH_4 473, - boc 356, -2 boc 256 (^{1}H)NMR (400 MHz, $CDCl_3$) 5.65 ppm (m, 1H), 5.45 ppm (m,1H), 4.79 ppm (m, 1H), 4.11 ppm (d, 2H), 3.68 ppm (s, 3H), 2.17 ppm (m, 4H), 1.47 ppm (s, 18 H).



Example U-7) Product from **Example U-6** (300 mg, 0.66 mmol) was dissolved in a solution of acetic acid and water (10 mL, 25/75) containing zinc metal and sonicated for 3 hours. The reaction mixture was filtered over celite and chromatographed on reverse phase HPLC to give a clear colorless residue (13 mg, 4 %).

(^{1}H)NMR (400 MHz, $CDCl_3$) 8.89 ppm (m, 1H), 5.68 ppm (m,1H), 5.47 ppm (m, 1H), 3.80 ppm (d, 2H), 3.71 ppm (s, 3H), 2.18 ppm (m, 4H), 1.41 ppm (s, 18 H).

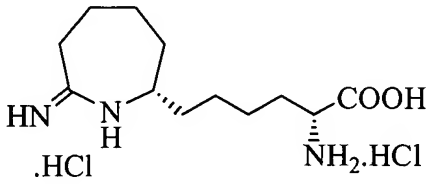
Example U) The product from **Example U-7** (13.0 mg, 0.031 mmol) was dissolved in 2 N HCl (1.22 mL, 2.44 mmol) and refluxed 1 hour. The reaction mixture was cooled, concentrated, to give a clear colorless oil (6.6 mg, 95%)

Mass Spectrometry: M+H 200,

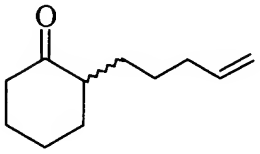
(^{1}H)NMR (400 MHz, D_20) 5.65 ppm (m, 1H), 5.47 ppm (m,1H), 3.80ppm (t, 1H), 3.72 ppm (d, 2H), 2.0 ppm (m, 5H), 1.87 ppm (m, 2H).

Example V:

(αR,2S)-α-aminohexahydro-7-imino-1H-azepine-2-hexanoic acid, trihydrate hydrochloride



Example V-1)

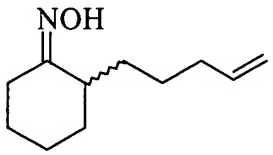


A three neck 3L flask was purged with nitrogen before it was charged with cyclohexanone (1.27 mol, 132 mL) and 500 mL of toluene. This stirred mixture was cooled to 0 °C and 157.2 g (1.1eq) of potassium t-butoxide was added. After stirring this mix for 1 hr, a color and texture change was noted before a

solution of 5-pentenyl bromide (1.27 mol, 136 mL) in 100 mL toluene was added dropwise over 1 h to the mechanically stirred reaction mixture. The reaction mixture was allowed to warm to 25 °C and stir overnight. It was then diluted with 800 mL of 1 N $KHSO_4$ and the organic phase was dried ($MgSO_4$), filtered and evaporated to dryness to yield 208.5 g of crude product. This material was then purified by vacuum distillation (under water aspirator pressure) to give the title product in 47% yield.

^{1}H NMR ($CDCl_3$, δ ppm): 1.0- 2.4 (m, 13H), 4.9-5.1 (m, 2H), 5.7-5.9 (m, 1H).

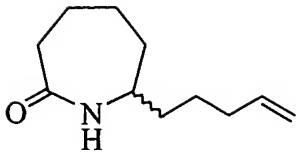
Example V-2)



The product of **Example V-1** (93.67 g, 0.563 mole) along with EtOH (600 mL), water (300 mL), NaOAc (101.67 g, 1.24 mole), and $NH_2OH.HCl$ (78.31 g, 1.13 mole) were combined in a three neck 3 L flask. This stirred reaction mixture was refluxed for 16 h and then stirred at 25 °C for another 24 h. All solvent was removed under reduced pressure and the residue was partitioned between diethylether (Et_2O, 500 mL) and water (200 mL). The aqueous layer was extracted 3 X 200 mL ether. The combined organic layers were dried over $MgSO_4$, filtered, and stripped in vacuo to give the title oxime (121.3 g, 100% crude yield).

^{1}H NMR ($CDCl_3$, δ ppm): 1.2- 2.6 (m, 13H), 4.9-5.1 (m, 2H), 5.7-5.9 (m, 1H).

Example V-3)



A three neck 3 L flask was purged with nitrogen and then charged with hexamethydisiloxane (471.7 mL, 2.2 moles), toluene (500 mL), and phosphorous pentoxide (203.88 g, 1.4 moles). This heterogeneous mixture was refluxed until a clear solution was obtained (about 1.5 h). After cooling this mixture to room temperature, the oxime product of **Example V-1** (102.1 g , 0.563 moles) in 200 mL of toluene was added to the above reaction mixture over a 1 h period at 25 °C. The reaction mixture was stirred for another 4 - 6 h (checked by TLC: 50% EA in Hex, I_2) before it was poured into ice water with thorough mixing. To this ice slurry mixture was added 250 g of NaCl and the resulting mixture was adjusted to pH 5 by adding solid potassium carbonate. This slurry was extracted with 3 X 500 mL of diethylether (Et_2O) and the combined organic fractions were dried over $MgSO_4$, filtered and stripped in vacuo to give the crude mixture of regioisomeric lactams (84.6 g).

Example V-4)

R-isomer + S-isomer

The product of **Example V-3** was then subjected to chromatography (silica: acetonitrile) for purification and regioisomeric separation. From the crude sample, the 7-pentenyl regioisomer was isolated in 50% yield and after chiral

chromatography, the desired single enantiomers were isolated in 43% yield each.

R-isomer:

Elemental analyses Calcd for $C_{11}H_{19}NO$: C, 71.99; H, 10.57; N, 7.63. Found: C, 71.97; H, 10.58; N, 7.52

^1H NMR ($CDCl_3$, δ ppm): 1.3-1.6 (m, 7H), 1.75-1.9 (m, 2H), 1.95-2.15 (m, 3H), 2.4-2.5 (m, 2H), 3.25-3.35 (m, 1H), 4.95-5.05 (m, 2H), 5.7-5.85 (m, 1H).

^{13}C NMR ($CDCl_3$, δ ppm): 23.166, 25.169, 29.601, 33.209, 35.475, 35.624, 36.783, 53.600, 114.976, 137.923, 177.703

$[\alpha]^{25}$ = +26.9° ($CHCl_3$) at 365nm.

S-isomer:

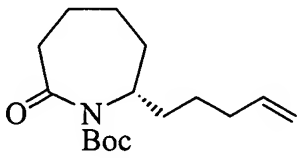
Elemental analyses Calcd for $C_{11}H_{19}NO$: C, 71.99; H, 10.57; N, 7.63. Found: C, 72.02; H, 10.61; N, 7.57

^1H NMR ($CDCl_3$, δ ppm): 1.3-1.6 (m, 7H), 1.75-1.9 (m, 2H), 1.95-2.15 (m, 3H), 2.4-2.5 (m, 2H), 3.25-3.35 (m, 1H), 4.95-5.05 (m, 2H), 5.7-5.85 (m, 1H).

^{13}C NMR ($CDCl_3$, δ ppm): 23.187, 25.178, 29.630, 33.230, 35.526, 35.653, 36.778, 53.621, 115.032, 137.914, 177.703

$[\alpha]^{25}$ = -25.7° ($CHCl_3$) at 365nm.

Example V-5)

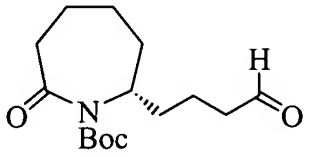


The R-isomer product of **Example V-4** (102.1 g, 0.56 mol), dry THF (800 mL), DMAP (68.9 g, 0.56 mol), Di-t-butyl dicarbonate (Boc_2O, 99 g, 0.45 mol)

were combined in a three neck 3L flask purged with argon. The reaction mixture was warmed to 70 °C within 30 min before an additional 52.8 g of Boc_2O and 200 mL of dry THF were added. After 30 min. another 32 g of Boc_2O was added and the mixture was stirred for 1 h at 70 °C. Another 36 g of Boc_2O was added and the mixture was stirred for 1 h. The reaction mixture was cooled to room temperature and stripped of THF at 18 °C to 20 °C under reduced pressure. A precipitate was filtered and washed with 100 mL of ethylacetate (EA) and discarded (~ 45 g). The EA filtrate was diluted with 500 mL of additional EA before it was washed with 500 mL of 1N $KHSO_4$, 500 mL of saturated aq. $NaHCO_3$, and 500 mL of brine and then dried over anhydrous Na_2SO_4 for 12 h. This EA extract was then treated with 20 g of DARCO, filtered through celite topped with $MgSO_4$, and concentrated *in vacuo* to give 150 g of title product as a dark brown oil.

^1H NMR ($CDCl_3$, δ ppm): 1.3-1.6 (m, 4H), 1.5 (s, 9H), 1.6-1.9 (m, 6H), 1.95-2.05 (m, 2H), 2.5-2.7 (m, 2H), 4.2-4.25 (m, 1H), 4.95-5.05 (m, 2H), 5.7-5.85 (m, 1H).

Example V-6)

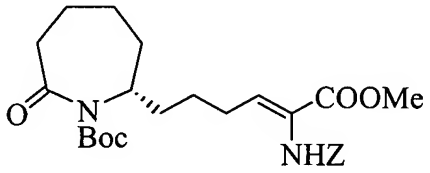


A three neck 3L flask containing the product of **Example V-5** (150 g, 0.533) dissolved in 3 L of CH_2Cl_2 was cool to -78 °C. A stream of O_3 was passed through the solution for 2.5 h until the color of the reaction mixture turned blue. Argon was then bubbled through the solution maintained at -60 °C to -70 °C until the solution became clear and colorless (~30 min.). Dimethylsulfide (DMS, 500 mL) was then added before the reaction was brought to reflux and

this reflux was continued for 24 h. Another 100 mL of DMS was added and reflux was continued for 12 h. Another 100 mL of DMS was added and reflux continued for an additional 12 h. The solvent and excess DMS were then stripped on a rotary evaporator at 20 °C. The residual yellow oil obtained was diluted with 500 mL of DI water and extracted with 3 X 300 mL of EA. The EA layer was dried over anhydrous $MgSO_4$, treated with 20 g of DARCO, filtered through a thin layer of celite topped with anhydrous $MgSO_4$, and stripped of all solvent under reduced pressure to yield 156 g of the crude title product as orange yellow oil.

^1H NMR ($CDCl_3$, δ ppm): 1.3-1.6 (m, 4H), 1.5 (s, 9H), 1.6-1.9 (m, 6H), 2.45-2.75 (m, 4H), 4.2-4.25 (m, 1H), 9.75 (s, 1H).

Example V-7)



To a sample of N-(Benzyloxycarbonyl)-alpha-phosphonoglycine trimethyl ester (160 g, 0.48 mol) dissolved in 1L of dichloromethane (CH_2Cl_2) and cooled to 0 °C was added a solution of DBU (110.29 g, 0.72 mol) in 100 mL of CH_2Cl_2. This clear colorless reaction mixture was stirred for 1h at 0 °C to 6 °C before the Boc-aldehyde product of **Example V-6** (150 g, 0.53 mol) in 600 mL of CH_2Cl_2 was added drop wise at -5 °C to -1 °C. The reaction mixture was stirred for 30 min. at this temperature before it was slowly warmed to 10 °C in

approximately 1 h. The reaction mixture was washed with 1N $KHSO_4$ (500 mL), saturated aq. $NaHCO_3$ (200 mL) and 50 aq. NaCl (200 mL). The organic layer was then dried over anhydrous $MgSO_4$, treated with 40 g of DARCO, filtered through a thin layer of celite topped with anhydrous $MgSO_4$, and concentrated to give 258 g of the crude title product as an yellow oil. Chromatographic purification of this material gave 130 g (55%) of the pure title product.

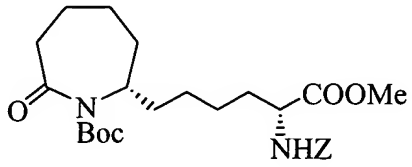
Elemental analyses Calcd for $C_{26}H_{36}N_2O_7$: C, 63.96; H,7.42; N, 5.77. Found: C, 63.42; H, 8.16; N, 5.31.

^{1}H NMR ($CDCl_3$, δ ppm): 1.25 (m, 2H), 1.5 (s, 9H), 1.51-1.9 (bm, 8H), 2.25 (m, 2H), 2.5 (m, 1H), 2.65 (m, 1H), 3.75 (s, 3H), 4.12 (m, 1H), 5.15 (s, 2H), 6.3 (bs, 1H), 6.55 (t, 1H), 7.45 (m,5H).

^{13}C NMR ($CDCl_3$, δ ppm): 14.04, 22.62, 23.46, 24.08, 25.27, 27.89, 27.92, 28.34, 28.95, 31.81, 31.86, 32.05, 39.18, 52.31, 54.65, 67.27, 82.62, 128.07, 128.18, 128.46, 135.98, 136.82, 154.50, 164.92, 176.68.

$[\alpha]^{25}$ = +10.9° ($CHCl_3$) at 365nm.

Example V-8)

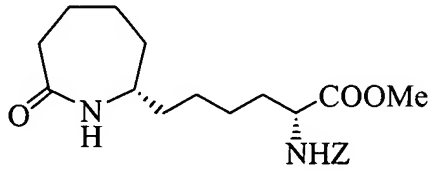


To a MeOH (1 L) solution of the product of **Example V-7** (91.3 g, 0.19 mol) was added 2.5 g of S,S-Rh-DIPAMP catalyst followed by hydrogen. The hydrogenation was carried out at 25 °C in 1.5 h in a Parr apparatus. The

reaction mixture was filtered through celite before concentrating to provide the crude title product (90 g, 98%) as a brown oil.

^{1}H NMR ($CDCl_3$, δ ppm): 1.35 (m, 4H), 1.5 (s, 9H), 1.55-1.95 (m, 10H), 2.4-2.7 (m, 2H), 3.75 (s, 3H), 4.2 (m, 1H), 4.4 (m, 1H), 5.1 (m, 2H), 5.35 (d, 1H), 7.35 (m, 5H).

Example V-9)



To a solution of the product of **Example V-8** (90 g,) in 200 mL of glacial acetic acid was

added 200 mL of 4N HCl in dioxane. The reaction mixture was stirred at 25 °C for 20 min. before it was stripped of all solvent under reduced pressure at 40 °C to give a red brown oil. This oily product was treated with 500 mL of water and extracted 2 X 300 mL of dichloromethane. The combined organic layer was washed with satd. sodium bicarbonate solution (100 mL), dried over magnesium sulfate, filtered and stripped of all solvent to give the crude title product. This material was chromatographed to provide 45 g (62%) of the pure title product.

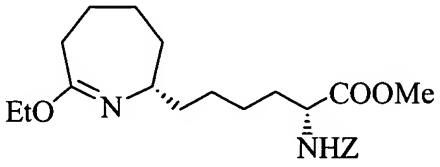
Elemental analyses Calcd for $C_{21}H_{30}N_2O_5$: C, 64.02; H, 7.68; N, 7.17. Found: C, 63.10; H, 7.88; N, 6.60.

^{1}H NMR ($CDCl_3$, δ ppm): 1.2-2.0 (m, 14H), 2.45 (t, 2H), 3.25 (m,1H), 3.75 (s, 3H), 4.38 (m, 1H), 5.1 (s, 2H), 5.3 (d, 1H), 5.45 (bs, 1H), 7.35 (m, 5H).

^{13}C NMR ($CDCl_3$, δ ppm): 14.09, 23.11, 24.89, 25.41, 29.53, 32.33, 35.52, 35.79, 36.68, 52.26, 53.51, 53.55, 53.60, 60.26, 66.86, 127.97, 128.05, 128.40, 136.18, 155.85, 172.85, 177.80.

$[\alpha]^{25}$ = -9.9° ($CHCl_3$) at 365 nm.

Example V-10)



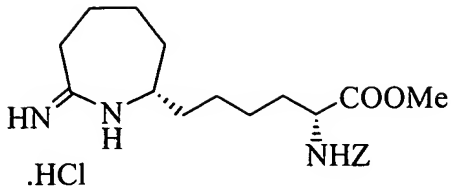
To a 45.0 g (0.115 mol) sample of the product of **Example V-9** in 300 mL of dichloromethane purged with argon was added 23.0 g (0.121 mol) of triethyloxonium tetrafluoroborate. This mixture was stirred for 1 h at 25 °C before 150 mL of satd. aq. sodium bicarbonate solution was added. The dichloromethane layer was separated, washed with 150 mL of 50% aq. NaCl solution, dried over sodium sulfate, filtered through celite and concentrated at 25 °C to give a clear yellow oil, 47.0 g (97%) of the title product

Elemental analyses Calcd for $C_{23}H_{34}N_2O_5$: C, 60.01; H, 8.19; N, 6.69. Found: C, 65.13; H, 8.45; N, 6.64.

^{1}H NMR ($CDCl_3$, δ ppm): 1.2 (t, 3H), 1.25-1.74 (m, 12H), 1.75-1.95 (m, 2H), 2.2-2.3 (m, 1H), 2.4-2.5 (m, 1H), 3.1 (m, 1H), 3.7 (s, 3H), 3.9-4.0 (m, 2H), 4.35 (m, 1H), 5.1 (s, 2H), 5.25 (d, 1H), 7.35 (m, 5H).

^{13}C NMR ($CDCl_3$, δ ppm): 14.23, 23.38, 25.01, 25.21, 26.10, 30.24, 32.16, 32.77, 33.92, 39.15, 52.22, 53.91, 58.05, 60.19, 66.92, 128.11, 128.33, 128.48, 136.27, 155.83, 166.29, 173.11, 177.64.

Example V-11)



To 7.0 g (0.130 mol) of ammonium chloride in 500 mL methanol was added 31.2 g of the title material of **Example V-10** (45.0 g, 0.107 mol). The reaction was refluxed at 65 °C for 5 h before all solvent was removed under reduced pressure to yield 40 g (87%) of the crude product as a foamy viscous mass. This material was purified by column chromatography to provide 37 g (81%) of the title product.

Elemental analyses Calcd for $C_{21}H_{31}N_3O_4$: C, 59.22; H, 7.57; N, 9.86; Cl, 8.32. Found for $C_{21}H_{31}N_3O_4$ + 1.2 HCl + 0.5 H_2O: C, 57.20; H, 7.99; N, 9.66; Cl, 9.62.
IR (Neat, λ max cm^{-1}): 2935, 1716, 1669.
^1H NMR ($CDCl_3$, □ ppm): 1.2-2.0 (m, 13H), 2.5 (t, 1H), 2.95 (m, 1H), 3.4 (bs, 1H), 3.7 (s, 3H), 4.3 (m, 1H), 5.1 (s, 2H), 5.55 (d, 1H), 7.3 (m, 5H), 8.75 (bs,1H), 8.9 (bs, 1H), 9.5 (s, 1H).
^{13}C NMR ($CDCl_3$, δ ppm): 23.20, 24.95, 25.22, 28.94, 31.80, 32.05, 33.75, 34.89, 52.33, 53.76, 56.07, 66.83, 127.93, 128.04, 128.43, 136.26, 156.00, 172.24, 172.87.
Mass (ESI): M/Z, 390.
$[\alpha]^{25}$ = +31.5° at 365 nm.

Example V)

The title product of **Example V-11** (36.0 g, 0.084 mol) in 1 L of 2.3 N HCl was refluxed for 3 h. After cooling to room temperature, the solution was washed with 2x150 mL of CH_2Cl_2 and then stripped of all solvent in vacuo to give 25.6 g (96%) of the title amino acid product as a pale yellow foam.

Elemental analyses Calcd for $C_{12}H_{23}N_3O_2.2HCl$: C, 46.02; H, 8.01; N, 13.39; Cl 22.45. Found for $C_{12}H_{23}N_3O_2$ + 2.2 HCl + 0.1 H_2O: C, 42.76; H,8.02; N, 12.41; Cl, 22.79.

IR (Neat, λmax, cm^{-1}): 2930, 2861, 1738,1665.

^1H NMR (CD_3OD, δ ppm): 1.3-2.5 (m, 16H), 2.6 (dd, 1H), 2.8 (t, 1H), 3.65 (m, 1H), 4.0 (t, 1H), 7.85 (s, 1H), 8.85 (s, 1H), 8.95 (s, 1H).

^{13}C NMR (CD_3OD, δ ppm): 24.49, 25.67, 26.33, 29.71, 31.26, 32.45, 35.04, 35.87, 53.73, 57.21, 171.77, 173.96.

UV, 282 nm, abs 0.015.

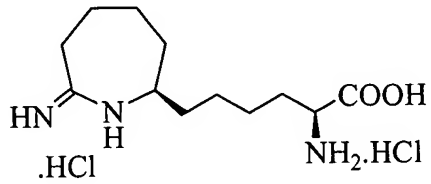
Mass (M^{+1}) = 242.

$[\alpha]^{25}$ = -47.4° (MeOH) at 365 nm.

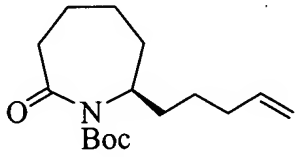
ee = 91% as determined by CE at λ = 214 nm.

Example W:

(αS,2R)-α-aminohexahydro-7-imino-1H-azepine-2-hexanoic acid, trihydrate hydrochloride



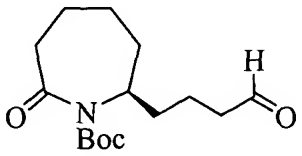
Example W-1)



The S-isomer product of **Example V-4** (5.45 g, 0.030 mol) was converted to its Boc derivative by the method of **Example V-5**. After chromatography, this reaction yielded 6.3 g (75%) of the desired title product.

^{1}H NMR ($CDCl_3$, δ ppm): 1.3-1.6 (m, 4H), 1.5 (s, 9H), 1.6-1.9 (m, 6H), 1.95-2.05 (m, 2H), 2.5-2.7 (m, 2H), 4.2-4.25 (m, 1H), 4.95-5.05 (m, 2H), 5.7-5.85 (m, 1H).

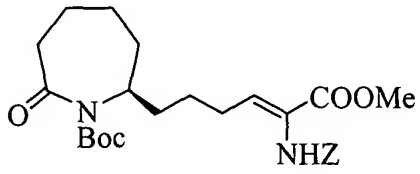
Example W-2)



The product of **Example W-1** (6.3 g, 0.025 mol) was ozonized by the method of **Example V-6** to produce 8.03 g of the crude title aldehyde that was used without further purification.

^{1}H NMR ($CDCl_3$, δ ppm): 1.3-1.6 (m, 4H), 1.5 (s, 9H), 1.6-1.9 (m, 6H), 2.45-2.75 (m, 4H), 4.2-4.25 (m, 1H), 9.75 (s, 1H).

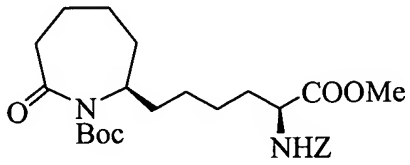
Example W-3)



The product of **Example W-2** (8.03 g, 0.024 mol) was condensed with N-(Benzyloxycarbonyl)-alpha-phosphonoglycine trimethyl ester (7.9 g, 0.024 mol) utilizing the procedure of **Example V-7** to produce 4.9 g (44%) of the desired title product after chromatography.

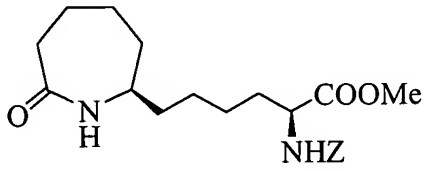
^{1}H NMR ($CDCl_3$, δ ppm): 1.25 (m, 2H), 1.5 (s, 9H), 1.51-1.9 (bm, 8H), 2.25 (m, 2H), 2.5 (m, 1H), 2.65 (m, 1H), 3.75 (s, 3H), 4.15-4.25 (m, 1H), 5.15 (s, 2H), 6.3-6.4 (bs, 1H), 6.45-6.55 (t, 1H), 7.3-7.4 (m,5H).

Example W-4)



The product of **Example W-3** (4.8 g, 0.010 mol) was reduced in the presence of R,R-Rh-DIPAMP catalyst by the method of **Example V-8** to produce 2.9 g (60%) of the desired title product after chromatography.

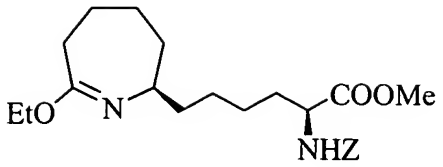
Example W-5)



The product of **Example W-4** (2.9 g, 0.006 mol) was deprotected by treatment with HCl using the method of **Example V-9** to produce 2.3 g (100%) of the desired title product.

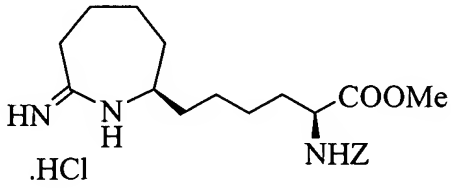
^{1}H NMR ($CDCl_3$, δ ppm): 1.3-2.0 (m, 14H), 2.45 (t, 2H), 3.25 (m,1H), 3.75 (s, 3H), 4.38 (m, 1H), 5.1 (s, 2H), 5.3 (d, 1H), 5.45 (bs, 1H), 7.35 (m, 5H).

Example W-6)



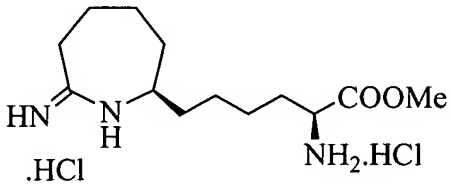
The product of **Example W-5** (0.56 g, 0.0015 mol) was alkylated with triethyloxonium tetrafluoroborate using the method of **Example V-10** to produce 0.62 g (98%) of the desired title product.

Example W-7)



The product of **Example W-6** (0.62 g, 0.0015 mol) was treated with ammonium chloride in methanol using the method of **Example V-11** to produce 0.50 g (88%) of the desired title product after chromatographic purification.

Example W-8)



The product of **Example W-7** (0.37 g, 0.0009 mol) dissolved in MeOH was added to a Parr hydrogenation apparatus. To this vessel was added a catalytic amount of 5%Pd/C. Hydrogen was introduced and the reaction was carried out at room temperature at pressure of 5 psi over a 7 hr period. The

catalyst was removed by filtration and all solvent was removed under reduced pressure from the filtrate to produce 0.26 g (quantitative) of the desired title product.

Example W)

A solution of the product of **Example W-8** dissolved in 2N HCl (30 mL) was maintained at reflux for 2 h before it was cooled to room temperature. All solvent was removed under reduced pressure and the residue was dissolved in 50 mL of water. This solution was again stripped of all solvent under reduced pressure before it was again dissolved in 12 mL of water and then lyophilized to generated 0.245 g (71%) of the title compound.

Elemental analyses Calcd for $C_{12}H_{23}N_3O_2$.2.3 HCl.1.9 H_2O: C, 40.10; H, 8.16; N, 11.69; Cl 22.69. Found for $C_{12}H_{23}N_3O_2$ + 2.1 HCl + 0.7 H_2O: C, 40.27; H, 8.28; N, 11.62; Cl, 22.70.

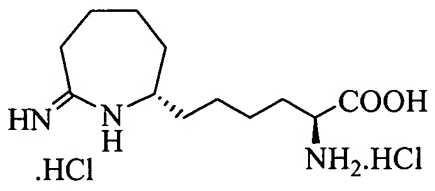
^{1}H NMR (CD_3OD, δ ppm): 1.4-2.1 (m, 16H), 2.6 (dd, 1H), 2.8 (t, 1H), 3.65 (m, 1H), 4.0 (t, 1H), 7.85 (s, 1H), 8.45 (s, 1H), 8.9 (s, 1H).

^{13}C NMR (CD_3OD, δ ppm): 24.46, 25.64, 26.31, 29.69, 31.24, 32.54, 35.00, 35.83, 53.75, 57.20, 171.85, 173.93.

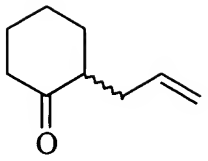
$[\alpha]^{25}$ = +25.7° (MeOH) at 365 nm.

Example X:

(αS,2S)-α-aminohexahydro-7-imino-1H-azepine-2-hexanoic acid, trihydrate hydrochloride



Example X-1)



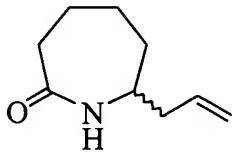
To a 22L round bottom flask equipped with overhead stirrer, half moon shape paddle, heating mantle, thermocouple, and a silver vacuum jacketed distillation column (5 plates) was charged cyclohexanone (4500.0 g, 45.85 mol), acetone dimethyl acetal (5252.6 g, 50.43 mol), allyl alcohol (6390.87 g, 110.04 mol) and p-toluene sulfonic acid (PTSA) (0.256 g, 0.001 mol). After the stirring was started (137 rpm) the pot was heated slowly with the initial set point being 70 °C. Heating was increased step wise to a final pot temperature of 150 °C. The decision to increase the reactor set point was made based on distillation rate. If the rate of distillate slowed or stopped, additional heat was applied. The additional heating to 150 °C allowed the Claisen rearrangement to occur. After the pot temperature was raised to 150 °C and no distillate was observed, the heating mantle was lowered and the reaction mixture allowed to cool to 130 °C. The PTSA was then neutralized with 3 drops of 2.5 N NaOH. The vacuum stripping was then started with the heating mantle lowered away from the flask. Evaporative cooling was used to lower the pot temperature, and the pressure was gradually lowered to 40 mm Hg. When the pot temperature had decreased to ~100 °C, the heating mantle was raised back into the proper position for heating. Unreacted cyclohexanone and low boiling impurities were distilled off. The pot temperature was slowly raised (the maximum temperature deferential between the pot and vapor was ~12 °C). The product was isolated at 109-112 °C @ 40 mm Hg. Typical yields were 40-45%. Fractions which were <95% by area (GC) were combined and redistilled to afford the title product in a total yield of 55%.

^{1}H NMR ($CDCl_3$, δ ppm): 5.8-5.6 (m, 1H), 4.8-5.0 (m, 2H), 2.5-2.4 (m, 1H), 2.3-2.1 (m, 3H), 2.1-1.2 (m, 7H).

^{13}C NMR ($CDCl_3$, δ ppm): 212.53, 136.62, 116.32, 50.39, 42.18, 33.91, 33.52, 28.09, 25.10.

GC/MS m/z = 138.

Example X-2)



Hydroxyl amine-O-sulfonic acid (91.8 g) dissolved in acetic acid (470 g) was added to a 1 L Bayer flask equipped with a mechanical stirrer, thermocouple, condenser chilled to 0 °C, and an addition funnel and heated to 70 °C. The allyl cyclohexone (100 g) was added dropwise in approximately 40 min to the above solution while maintaining the temperature between 70 and 78 °C. During the addition, the reaction appearance changed from a white slurry to a clear orange solution. After the addition, the reaction was heated and stirred for an additional 5 h at 75 °C. An IPC sample was taken each hour. After the reaction was complete, the acetic acid was stripped at 50 °C under reduced pressure on a rotary evaporator. Water (200 mL) was then added to the residue and the solution extracted with toluene (2 X 300 mL). The organic layers were combined, treated with water (150 ml) and stirred for 10 min. A sodium hydroxide solution (79.4 g of 50 solution) was added until the aqueous layer turned basic (pH 12). The neutralization was carried out in the reactor by controlling the temperature below 40 °C. The layers were then separated and the toluene layer was passed through a filter to remove any solids or tarry material. The organic solution was then stripped at 50 °C under

reduced pressure on a rotary evaporator. The residue was taken up in a mixture of toluene (510 mL) and heptanes (2040 mL) and heated to 60 °C in a 3 L reactor. A clear yellow-orange solution was obtained. The title product began to crystallize at 53 °C as the solution was slowly cooled to 5 °C while being stirred. The solid was filtered, washed with heptanes (50 mL) and dried over night at 40 °C under house vacuum to produce 66.3 g (60%) of title product as off-white crystals obtained. A portion of this material was recrystallized from toluene and heptane to generate the title product as a white crystalline solid.

^{1}H NMR ($CDCl_3$, δ ppm): 5.8-5.6 (m, 1H), 5.5 (bs, 1H), 4.8-5.0 (m, 2H), 3.4-3.3 (m, 1H), 2.5-2.3(m, 2H), 2.3-2.1 (m, 2H) 2.0-1.2 (m, 6H)

^{13}C NMR ($CDCl_3$, δ ppm): 117.73, 133.83, 119.31, 52.88, 40.95, 37.20, 35.75, 29.96, 23.33.

GC/MS (EI mode) = 153.

m.p. = 97-99 °C.

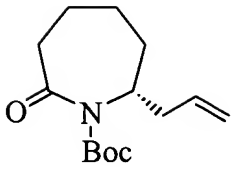
Example X-3)

R-isomer + S-isomer

The racemic product mixture of **Example X-2** was subjected to chiral chromatographic separation on a Chiralpac AS 20 um column eluting with 100% acetonitrile. A 220 nM wavelength was employed in the detector. A sample loading of 0.08 g/mL of acetonitrile was used to obtain 90% recovery of separated isomers each with >95% ee. A portion of the R-isomer material was recrystallized from toluene and heptane to generate the R-isomer title product as a white crystalline solid.

R-isomer: m.p. = 81 - 82 °C.

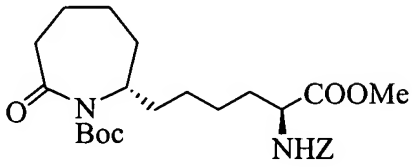
Example X-4)



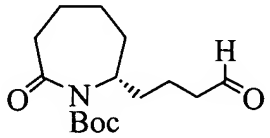
A five necked flat bottom flask equipped with dropping funnel, thermometer and mechanical overhead stirrer was evacuated and purged with nitrogen three times. The R-isomer product lactam of **Example X-3** (100.0 g, 0.653 mol), DMAP (7.98 g, 65 mmol) and *N*-diisopropylethyl amine (Hünigs base, 113.3 g, 0.876 mol) were dissolved in toluene (350 mL) and Di-tert-butyl dicarbonate (170.2 g, 0.78 mol) dissolved in toluene (100 mL) was added. (Note: the reaction works better, when 2.0 eq of Hünigs base were used). The mixture was heated to 65 °C (Note: Steady offgasing during the reaction was observed). After 1.5 h another 86.25 g of Di-tert-butyl-dicarbonate (0.395 mol) dissolved in toluene (50 mL) were added. Heating was continued for 17 h and IPC by HPLC showed 75 conversion. Another 42.78 g of Di-tert-butyl dicarbonate (0.196 mol) in toluene (30 mL) were added and the brown mixture was heated 5.5 h. After cooling to ambient temperature, the mixture was treated with 4M HCl (215 mL), and the aqueous layer was extracted with toluene (2x80 mL). The combined organic layers were washed with $NaHCO_3$ (170 mL) and 250 ml of water (Note: the internal temperature during the quench was controlled by external cooling with ice/water). Gas evolution was observed. The organic layer was evaporated to give 257.4 g brown liquid. This crude material was purified by plug filtration over SiO_2 (950 g) using

toluene / EtOAc 9/1 (6 L) and toluene/AcOEt 1/1 (0.5 L) as eluent giving 139.5 g (51%) of the yellow liquid title product.

Example X-5)



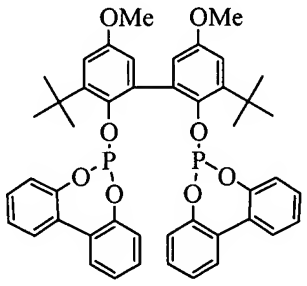
Example X-6)



Example 1f

Into a 2-L stainless steel autoclave equipped with baffles and a six-bladed gas dispersing axial impeller was charged $Rh(CO)_2(acac)$ (0.248 g, 0.959 mmol), BIPHEPHOS (structure shown below and prepared as described in Example 13 of US patent 4,769,498, 2.265 g, 2.879 mmol), the product of **Example X-4** (*N*-(*tert*-butoxycarbonyl)-S-7-allylcaprolactam

BIPHEPHOS

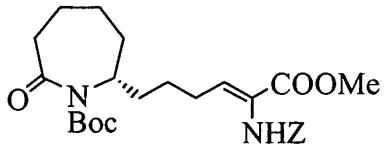


(242.9 g, 0.959 mol), and toluene (965 g). The reactor was sealed and purged 100% carbon monoxide (8 × 515 kPa). The reactor was pressurized to 308

kPa (30 psig) with 100% carbon monoxide and then a 1:1 CO/H_2 gas mixture was added to achieve a total pressure of 515 kPa (60 psig). With vigorous mechanical agitation, the mixture was heated to 50 °C with a 1:1 CO/H_2 gas mixture added so as to maintain a total pressure of about 515 kPa (60 psig). After 22 h, the mixture was cooled to about 25 °C and the pressure was carefully released. Vacuum filtration of the product mixture and evaporation of the filtrate under reduced pressure afforded a 267.7 g of a light yellow oil. Analysis by ^1H NMR was consistent with essentially quantitative conversion of the starting material with about 96% selectivity to the corresponding aldehyde product of **Example V-6**. This oil was used without further purification in the following example.

^1H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.6-1.80 (m, 9H), 1.84-1.92(m, 1H), 2.41-2.58 (m, 3H), 2.61-2.71 (m, 1H), 4.2 (d, *J* =5.2 Hz, 1H), 9.74 (s, 1H).

Example X-8)



Example 1g

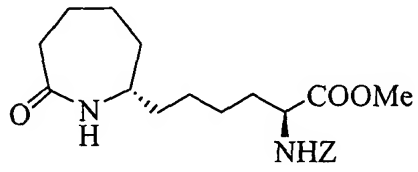
To a sample of N-(Benzyloxycarbonyl)-alpha-phosphonoglycine trimethyl ester (901.8 g, 2.7 mol) dissolved in CH_2Cl_2 and cooled to 0 °C was added a solution of DBU (597.7 g, 3.9 mol) in CH_2Cl_2. This clear colorless reaction mixture was stirred for 1h at 0 °C to 6 °C before a sample of the Boc-aldehyde product **Example V-6** (812.0 g, 2.9 mol) in CH_2Cl_2 was added drop wise at -5 °C to -1 °C. The reaction, work up, and purification was completed as described in **Example V-7** to give 1550 g of the title product of **Example V-7** containing a small amount of CH_2Cl_2.

Example X-9)

To a MeOH (1 L) solution of the product of **Example V-7** (100 g, 0.20 mol) was added 3 g of RR-Rh-DIPAMP catalyst. The hydrogenation was carried out at 25 °C in 1.5 h in a Parr apparatus. The reaction mixture was filtered through celite before concentrating to provide the crude **Example X-9** title product as a brown oil (100 g).

^{1}H NMR ($CDCl_3$, δ ppm): 1.35 (m, 4H), 1.5 (s, 9H), 1.6-1.9(m, 10H), 2.5-2.8 (m, 2H), 3.75 (s, 3H), 4.25 (m, 1H), 4.45 (m, 1H), 5.1 (m, 2H), 5.65 (d, 1H), 7.35 (m, 5H).

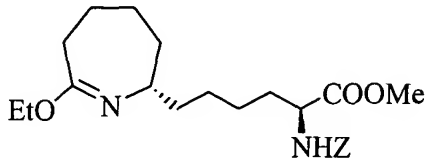
Example X-10)



To a solution of the product of **Example V-8** (100 g) in 200 mL glacial acetic acid was
added 25 mL 4N HCl in dioxane. The reaction mixture was stirred at 25 °C for 20 min. before it was stripped of all solvent under reduced pressure at 40 °C to give 105 g of red brown oil. This oily product was treated with 500 mL of water and extracted 2 X 300 mL of dichloromethane. The combined organic layer was washed with satd. sodium bicarbonate solution (100 mL), dried over magnesium sulfate, filtered and stripped of all solvent to give 99.9 g of the title product as a red brown oil.

^{1}H NMR ($CDCl_3$, δ ppm): 1.25-2.0 (m, 14H), 2.45 (t, 2H), 3.25 (m,1H), 3.7 (s, 3H), 4.35 (m, 1H), 5.1 (s, 2H), 5.5 (d, 1H), 6.45 (bs, 1H), 7.35 (m, 5H).
ee = 95% as determined by chiral HPLC.

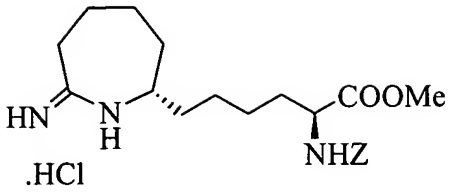
Example X-11)



To a 30.0 g (0.077 mol) sample of the product of **Example X-10** in 600 mL dichloromethane purged with argon was added 15.7 g (0.082mol) of triethyloxonium tetrafluoroborate. This mixture was stirred for 1 h at 25 °C before 300 mL of satd. aq. sodium bicarbonate solution was added. The dichloromethane layer was separated, washed with 300 mL 50% aq. NaCl solution, dried over sodium sulfate, filtered through celite and concentrate at 25 °C to give a clear yellow oil, 31.2 g (~97%) of the title product.

Elemental analyses Calcd for $C_{23}H_{34}N_2O_5$: C, 60.01; H, 8.19; N, 6.69. Found for $C_{23}H_{34}N_2O_5$ + 0.5 H_2O: C, 64.66; H, 8.24 N,6.59.
^{1}H NMR ($CDCl_3$, δ ppm): 1.2 5(t, 3H), 1.28-1.75 (m, 12H), 1.8-1.98 (m, 2H), 2.2-2.3 (m, 1H), 2.4-2.5 (m, 1H), 3.1 (m, 1H), 3.78 (s, 3H), 3.9-4.0 (m, 2H), 4.35 (m, 1H), 5.1 (s, 2H), 5.25 (d, 1H), 7.35 (m, 5H).
^{13}C NMR ($CDCl_3$, δ ppm): 14.27, 23.36, 25.21, 25.53, 26.09, 30.22, 32.15, 32.73, 33.90, 39.14, 52.21, 53.89, 58.04, 60.33, 66.89, 128.11, 128.35, 128.48, 136.29, 155.86, 166.30, 173.14, 177.69.
IR (Neat, λmax, cm^{-1}): 3295, 2920, 1739, 1680.
UV , 257 nm, abs 0.015.
$[\alpha]^{25}$ = +39.8° ($CHCl_3$) at 365 nm.

Example X-12)



To 4.2 g (0.078 mol) of ammonium chloride in 500 mL methanol was added 31.2 g of the title material of **Example X-11**. The reaction was refluxed at 65 °C for 5 h before all solvent was removed under reduced pressure to yield 29 g (92%) of the crude product as a foamy viscous mass. This material was purified by column chromatography to provide 23 g (70%) of the title product.

Elemental analyses Calcd for $C_{21}H_{31}N_3O_4$.1HCl) C, 59.28; H, 7.57; N, 9.89; Cl, 8.39. Found (For $C_{21}H_{31}N_3O_4$ + 1HCl + 1 H_2O): C, 56.73; H, 7.74; N, 9.40; Cl, 8.06.

IR (Neat, λ max cm^{-1}): 3136, 30348, 2935, 1716, 1669.

^1H NMR ($CDCl_3$, δ ppm): 1.3-2.05 (m, 13H), 2.5 (t, 1H), 2.98 (m, 1H), 3.4 (bs, 1H), 3.75 (s, 3H), 4.35 (m, 1H), 5.1 (s, 2H), 5.5 (d, 1H), 7.35 (m, 5H), 8.75 (s,1H), 9.0 (s, 1H), 9.5 (s, 1H).

^{13}C NMR ($CDCl_3$, δ ppm): 23.25, 25.01, 25.34, 29.01, 31.88, 32.26, 33.89, 35.06, 52.33, 53.73, 56.20, 66.89, 127.95, 128.06, 128.45, 136.27, 155.93, 172.27, 172.80.

UV , 257 nm, abs 0.009.

Mass (ESI): M/Z, 390.

$[\alpha]^{25}$ = -42.8° (MeOH) at 365 nm.

ee = 96% as determined by chiral HPLC.

Example X)

The title product of **Example X-12** (23 g) in 500 mL 2N HCl was refluxed for 5 h. All solvent was then removed in vacuo and the residue redissolved in water was washed with 2x300 mL of CH_2Cl_2. The aqueous was then concentrated *in vacuo* to give 17 g (100%) of the light brown hygroscopic solid title product.

Elemental analyses Calcd for $C_{12}H_{23}N_3O_2.2HCl$: C, 45.86; H, 8.02; N, 13.37; Cl 22.56. Found for $C_{12}H_{23}N_3O_2$ + 2.1 HCl + 0.7 H_2O: C, 43.94; H, 8.65; N, 12.52; Cl, 22.23.

IR (Neat, λmax, cm^{-1}): 2936, 1742,1669.

^1H NMR (CD_3OD, δ ppm): 1.3-2.1 (m, 16H), 2.6 (dd, 1H), 2.8 (t, 1H), 3.65 (m, 1H), 4.0 (t, 1H), 7.85 (s, 1H), 8.4 (s, 1H), 8.95 (s, 1H).

^{13}C NMR (CD_3OD, δ ppm): 24.49, 25.67, 26.33, 29.71, 31.26, 32.45, 35.04, 35.87, 53.73, 57.21, 171.77, 173.96.

UV, 209 nm, abs 0.343.

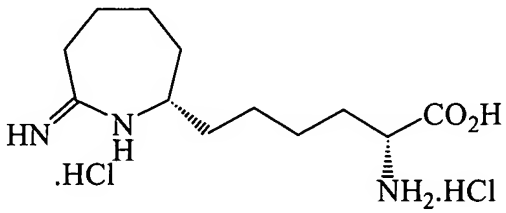
Mass (M^{+1}) = 242.

$[\alpha]^{25}$ = +60.0° (MeOH) at 365 nm.

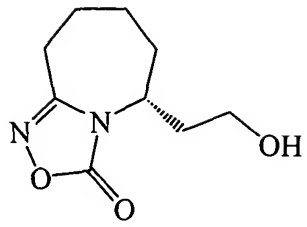
ee = 92% as determined by CE at λ = 210 nm.

Example Y

(αR,2S)-α-aminohexahydro-7-imino-1H-azepine-2-hexanoic acid, trihydrate hydrochloride



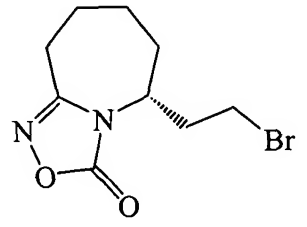
Example Y-1)



A solution of **Example X-3** (3.0g, 0.015 mol) in methylene chloride and methanol (75/45 mL) was cooled to -78 °C in a dry ice bath. The reaction stirred as ozone was bubble through the solution at a 3ml/min flow rate. When the solution stayed a consistent deep blue, the ozone was remove and the reaction was purged with nitrogen. To the cold solution was added sodium borohydride (2.14 g, .061 mol) very slowly to minimize the evolution of gas at one time. To the reaction was added glacial acetic acid slowly to bring the pH to 3. The reaction was then neutralized with saturated sodium bicarbonate. The oraganics were then washed 3x 50mL with brine, dried over magnesium sulfate anhydrous, removed under reduced pressure. The pale oil was run through a plug of silica (15 g) to afford the alcohol 5.15 g, 0.026 mol (64 %). $C_9H_{14}N_2O_3$.

^{1}H NMR ($CDCl_3$, δ ppm) 1.18 - 2.15(m, 8H), 3.59(m, 2H), 4.39(m, 1H).
^{13}C NMR ($CDCl_3$, δ ppm) 24.45, 25.71, 26.47, 32.56, 34.67, 51.16, 58.85, 160.66, 160.89.

Example Y-2)



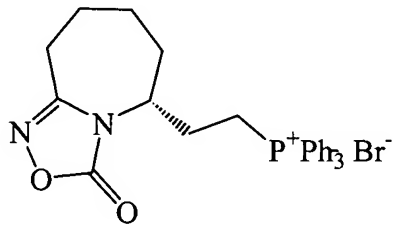
To a solution of **Example Y-1** (5.15 g, 0.026 mol) in methylene chloride (100 mL) at 0 °C in an ice bath was added carbon tetrabromide(10.78 g, 0.033 mol) . The solution was cooled to 0 °C in an ice bath. Then triphenylphosphine (10.23 g, 0.39 mol) was added portion wise as not to allow the temperature raise above 3 °C. The reaction was stirred for 2 hours and the solvent was removed in vacuo. The crude was purified by flash chromatography to yield the bromide (5.9 g, 0.023 mol) in 87% yield.

Elemental analysis calculated for $C_{10}H_{16}N_2O_3$: C, 41.40; H, 5.02; N, 10.73; Br, 30.60. Found: C, 41.59; H, 5.07; N, 10.60, Br, 30.86.

^{1}H NMR ($CDCl_3$, δ ppm) 1.50 - 2.60 (m, 9H), 2.99 (dd, 1H), 3.35 (m, 2H), 4.41 (m, 1H).

^{13}C NMR ($CDCl_3$, δ ppm) 23.89, 25.33, 26.04, 28.06, 31.59, 35.05, 52.79, 159.3, 160.2.

Example Y-3)



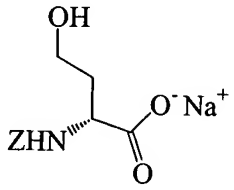
To a solution of **Example Y-2** (5.71 g, 0.026 mol) in toluene (25 mL) was added triphenyl phosphine (7.17 g, 0.027 mol). The reaction refluxed in an oil bath for 16 hours. After cooling, the toluene was decanted from the glassy solid. The solid was triturated with diethyl ether overnight to afford the phosphonium bromide (10.21 g, 0.020 mol) in 90% yield.

^{1}H NMR ($CDCl_3$, δ ppm): 1.50 - 2.9 (m, 11H), 3.58 (m, 1H), 4.16 (m, 1H), 4.41 (m, 1H), 7.6-8.0 (m, 15H).

^{13}C NMR ($CDCl_3$, δ ppm): 24.43, 24.97, 25.50, 55.08, 55.27, 116.9, 118.1, 130.4, 130.6, 133.5, 135.1, 135.2, 159.4, 160.

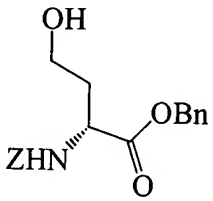
^{31}P NMR ($CDCl_3$, δ ppm) 26.0.

Example Y-4)



To a 1L Round Bottom Flask was added N-benzyloxycarbonyl-D-homoserine lactone (97 g, 0.442 mol) in ethanol (500 mL). To the reaction was added solution of sodium hydroxide (1M, 50mL). The reaction was monitored by thin layer chromatography for 12 hours until the starting material had been consumed. Toluene (60 mL) was added and then solvent was removed in vacuo. The residue was carried on with no further purification.

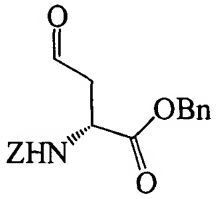
Example Y-5)



The residue from **Example Y-4** was suspended in DMF in a 1L Round Bottom Flask. To the suspension was added benzyl bromide (76.9 g, 0.45 mol, 53.5 mL) and the mixture was stirred for 1 hour. A sample was quenched and analyzed by mass spec to indicate the consumption of the starting material and that there was no lactone reformation. To the reaction was added

1L of ethyl acetate and 500 mL of brine. The aqueous layer was washed 2 additional times with 500 mL of ethyl acetate. The organics were combined, dried over $MgSO_4$ and concentrated. Silica gel chromatography provided N-benzyloxycarbonyl-S-homoserine benzyl ester as a white solid (80 g).

Example Y-6)

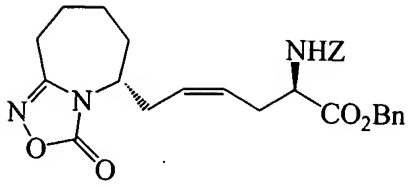


To a 2L Round Bottom Flask was added pyridinium chlorochromate (187 g, 0.867 mol) and silica gel (197 g) suspended in CH_2Cl_2 (600 mL). To the slurry was added a solution of the product of **Example Y-5** (80 g, 0.233 mol) in CH_2Cl_2 (600 mL). The mixture was stirred for 4 hours. Thin layer chromatography indicated that the starting material was consumed. To the reaction was added 1 L of diethyl ether. The solution was then filtered through a pad of ceilite followed by a pad of silica gel. The solvent was removed in vacuo and the resulting oil was purified by silica gel chromatography to afford the aldehyde (58.8 g) in 38% overall yield.

MH^+342.5, $MH+NH_4^+$359.5.

^1H NMR ($CDCl_3$, δ ppm) 3.15 (q, 2H), 4.12 (m, 1H), 5.15 (s, 2H), 5.20 (s, 2H), 7.31 (m, 10H), 9.72 (s,1H).

Example Y-7)



To a 3L 3-neck flask was added the phosphonium salt from **Example Y-3** (56.86 g, 0.11 mol) that had been dried over P_2O_5 under a vacuum in THF (1L). The slurry was cooled to -78 °C in a dry-ice bath. To the cold slurry was added KHMDS (220 mL, 0.22 mol) dropwise so that the temperature did not rise above -72 °C. The reaction was stirred at -78 °C for 20 minutes and then -45 °C for 2 hours. The temperature was then dropped back to -78 °C and the aldehyde (15.9 g, 0.047 mol) from **Example Y-6** was added in THF (50 mL) dropwise over 45 minutes. The reaction was stirred at -77 °C for 30 minutes then warmed to -50 °C for 1 hour before it was warmed to room temperature over 4 hours. To the reaction was added ethyl acetate (200 mL) and saturated ammonium chloride. The organics were collected, dried over $MgSO_4$ and concentrated in vacuo. The crude oil was purified on silica chromatography to afford the olefin compound (45.1 g) in 81% yield as a pale yellow viscous oil.

^1H NMR ($CDCl_3$, δ ppm) 1.4-2.6 (m,.10H), 2.92(d, 1H), 4.17(m, 1H), 4.38(m, 1H), 5.05(q, 2H), 5.40(m, 2H), 7.3(m,10H).

^{13}C NMR ($CDCl_3$, δ ppm) 29.49, 29.64, 31.32, 39.60, 49.56, 53.98, 61.01, 65.25, 124.14, 127.81, 128.20, 128.55, 128.79, 129.30, 130.96, 135.68, 137.31, 152.59, 157.57, 171.61.

Example Y)

To a 20 mL vial was added the product from **Example Y-7** (19.77 g, 0.039 mol) in Dioxane (50 mL) and 4N aqueous HCl (250 mL). This solution was added a cat. amount of 10% Pd on carbon in a hydrogenation flask. The flask was pressurized with H_2 (50 psi) for five hours. The reaction was monitored by mass spec and the starting material had been consumed. The solution was filtered through a pad of celite and washed with water. The solvent was removed by lyophollization to afford the title compound (7.52 g) in 81% yield.

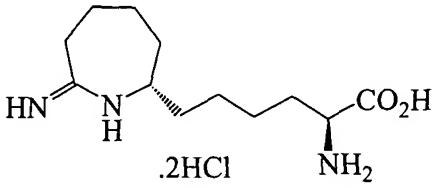
MH^+ 242.2, $MH+NH_4^+$ 259.2.

^1H NMR (CD_3OD δ ppm) 1.2-2.0 (m, 15H), 2.42 (d, 1H), 2.65 (dd, 1H), 3.49 (m, 1H), 3.98 (t, 1H), 7.26 (s), 8.05 (s), 8.35 (s).

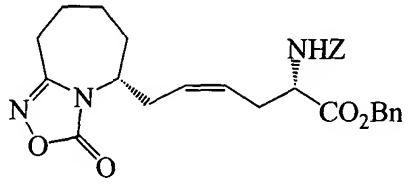
^{13}C NMR ($CDCl_3$, δ ppm) 24.43, 25.58, 26.00, 26.10, 32.75, 33.45, 35.31, 53.76, 54.55, 157.27, 175.13.

Example Z

(αS,2S)-α-aminohexahydro-7-imino-1H-azepine-2-hexanoic acid, trihydrate hydrochloride



Example Z-1)



To a 1 L 3-neck flask was added the phosphonium salt from **Example Y-3** (21.21g, 0.041 mol) in THF (200 mL). The slurry was cooled to -78 °C in a dry-

ice bath. To the cold slurry was added KHMDS (88 mL, 0.044 mol) dropwise so that the internal temperature did not rise above -72 °C. The reaction stirred at -78 °C for 20 minutes then -45 °C for 1 hour. The temperature was then dropped back to-78 °C and the aldehyde (15.9 g, 0.047 mol) (prepared as in **Example Y(4-6)** using N-benzyloxycarbonyl-L-homoserine lactone) was added in THF (50 mL) dropwise over 45 minutes. The reaction was stirred at -77 °C for 30 minutes then warmed to -50 °C for 30 minutes then warmed to room temperature over 4 hours. To the reaction was added ethyl acetate (100 mL) and saturated ammonium chloride. The organics were collected, dried over $MgSO_4$ and concentrated in vacuo. The crude oil was purified on silica chromatography to afford the olefin compound (9.0 g) in 45% yield as a pale yellow viscous oil.

^{1}H NMR ($CDCl_3$, δ ppm) 1.4-2.6 (m, 10H), 2.92 (d, 1H), 4.17 (m, 1H), 4.38 (m, 1H), 5.05 (q, 2H), 5.40 (m, 2H), 7.3 (m,10H).
^{13}C NMR ($CDCl_3$, δ ppm) 29.49, 29.64, 31.32, 39.60, 49.56, 53.98, 61.01, 65.25, 124.14, 127.81, 128.20, 128.55, 128.79, 129.30, 130.96, 135.68, 137.31, 152.59, 157.57, 171.71.

Example Z)

To a 20 mL vial was added the product from **Example Z-1** in dioxane (5 mL) and 4N aqueous HCl (16 mL). This solution was added a cat. amount of 10% Pd on carbon in a hydrogenation flask. The flask was pressurized with H_2 (50 psi) for five hours. The reaction was monitored by mass spec and the starting material had been consumed. The solution was filtered through a pad of ceilite and washed with water. The solvent was removed by lyophilization to afford the title compound (98.7mg) in 79.4% yield.

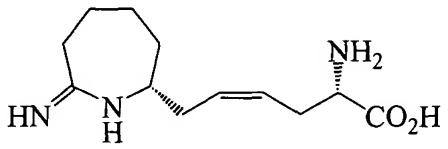
MH^+ 242.2, MH+$NH4^+$ 259.2.

^1H NMR (CD_3OD, δ ppm) 1.2-2.0 (m, 15H), 2.42 (d, 1H), 2.6 (dd, 1H), 3.49 (m, 1H), 3.98 (t, 1H).

^{13}C NMR ($CDCl_3$, δ ppm) 24.43, 25.58, 26.00, 26.10, 32.75, 33.45, 35.31, 53.76, 54.55, 157.27, 175.13.

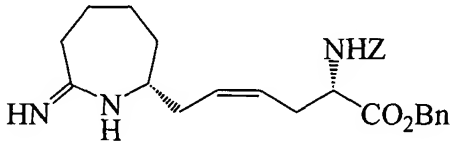
Example AA

(2*S*,4*Z*)-2-amino-6-[(2*R*)-hexahydro-7-imino-1*H*-azepin-2-yl]-4-hexenoic acid



Example AA-1)

(2*S*,4*Z*)-6-[(2*R*)-hexahydro-7-imino-1*H*-azepin-2-yl]-2-[[(phenylmethoxy)carbonyl]amino]-4-hexenoic acid, phenylmethyl ester



To a 50 mL flask was added a sample of **Example Z-1** (1.5g, 2.97 mmol) in methanol (25mL). A 60% solution of glacial acetic acid (16 mL) was then added to the reaction mixture. A precipitate was observed. Additional methanol was added to dissolve the solid (1mL). To the reaction was then added zinc dust (0.200g). The reaction was sonicated for 4 hours during which the temperature was maintained at 37 °C. The reaction was monitored by TLC and MS until the starting material was consumed and a mass corresponding to the product was observed. The solution was decanted from the zinc and a 30% solution of acetonitrile/water (100 mL) was added to the

filtrate. The reaction was purified with 52% acetonitrile/water in two runs on the Waters Preparatory HPLC [a gradient of from 20% to 70% acetonitrile over 30 minutes]. Lyophilization of the resulting product afforded the title material of **Example AA-1** (1.01g) in 73% yield as a white solid.

MH^+ 464.4, $MH+Na^+$ 486.4.

^1H NMR (CD_3OD, δ ppm): 1.2-2.0 (m, 8H), 2.42 (m, 2H), 2.6 (m, 5H), 3.49 (q, 1H), 4.31 (t, 1H), 5.15 (s, 2H), 5.22 (s, 2H), 5.43 (q, 1H), 5.59(q, 1H), 7.25 (bs, 10H).

^{13}C NMR ($CDCl_3$, δ ppm): 24.37, 29.61, 30.76, 32.45, 33.73, 34.42, 55.40, 57.09, 68.06, 68.07, 122.3, 124.9, 128.76, 129.09, 129.28, 129.39, 129.51, 129.61, 155.71, 158.35, 173.90.

Example AA)

To a 250 mL flask was added the product of **Example AA-1** (1.0g, 2.2mmol) in 4 M HCl (100mL). The reaction was refluxed overnight, monitored by MS until the starting material had been consumed and the mass for the product was observed. The reaction, without further work up was purified in two runs on the Water's prep reverse phase column using 18% acetonitrile/water [0% to 30% acetonitrile/water over 30 minutes]. Lyophilization of the combined fractions afforded the title product (0.34g) in 64% yield as a cream colored foam.

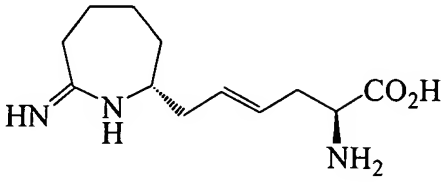
MH^+ 240.3, $MH+Na^+$ 486.4.

^1H NMR (CD_3OD, δ ppm): 1.2-2.0 (m, 6H), 2.35 (m, 2H), 2.45 (dd, 2H), 2.69 (m, 2H), 3.61 (dt, 1H), 3.98 (t, 1H), 5.59(m, 1H), 5.65 (m, 1H).

^{13}C NMR ($CDCl_3$, δ ppm): 23.65, 24.66, 32.51, 32.84, 33.1, 33.25, 54.10, 56.1, 126.80, 129.33, 153.33, 172.52.

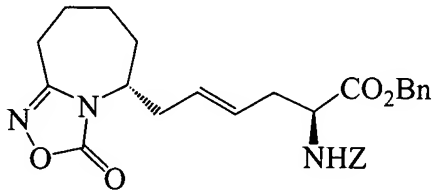
Example BB

(2*S*,4*E*)-2-amino-6-[(2*R*)-hexahydro-7-imino-1*H*-azepin-2-yl]-4-hexenoic acid



Example BB-1)

(2*S*,4*E*)-2-[[(phenylmethoxy)carbonyl]amino]-6-[(5*R*)-6,7,8,9-tetrahydro-3-oxo-3*H*,5*H*-[1,2,4]oxadiazolo[4,3-*a*]azepin-5-yl]-4-hexenoic acid, phenylmethyl ester



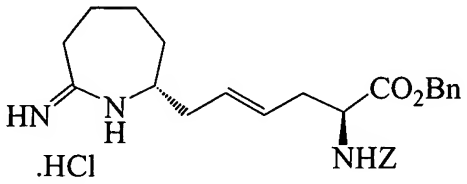
To a 250 mL flask was added **Example Z-1** (2.0g, 3.9 mmol) and phenyl disulfide (0.860g, 3.9mmol) in a cyclohexane (70mL) / benzene(40mL) solution. Nitrogen was bubbled through the solution to purge the system of oxygen. The reaction was exposed to a short wave UV lamp for the weekend. The reaction was evaluated by normal phase HPLC (ethyl acetate/hexane). 71% of the trans isomer and 29% of the cis isomer was observed. The reaction was subjected to an additional 3 days of UV upon which 84% of the starting material converted to the trans isomer and 16% of the starting cis isomer remained. Purification by chromatography afforded **Example BB-1** (0.956g) in 48% yield.

MH^+ 506.1, MH+$NH4^+$ 523.2.

^{1}H NMR (CD_3OD, δ ppm): 1.2-2.0 (m, 8H), 2.42 -2.6 (m, 6H), 2.91 (dd, 1H), 4.19 (m, 1H), 4.31 (dt, 1H), 5.09 (s, 2H), 5.11 (s, 2H), 5.18 (dt, 1H), 5.27(m, 1H), 7.25 (bs, 10H).

Example BB-2)

(2*S*,4*E*)-6-[(2*R*)-hexahydro-7-imino-1*H*-azepin-2-yl]-2-[[(phenylmethoxy)carbonyl]amino]-4-hexenoic acid, phenylmethyl ester, monohydrochloride



A sample of the product of **Example BB-1** (0.956g, 1.9mmol) in MeOH (80mL) was deprotected by method of **Example AA-1** with Zn dust (1.5g) and 60% HOAc/H_2O (40 mL). The resulting product was purified by reverse phase chromatography to afford the title material (0.248g) in 28% yield.

Example BB)

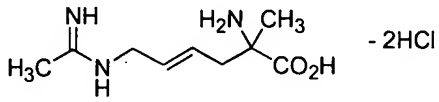
The product of **Example BB-2** (0.248g, 0.53mmol) was transformed into the title product by the method of **Example AA** using HCl (2mL), H_2O (2mL), CH_3CN (4mL). The crude product was purified by reverse phase chromatography to afford the title product of **Example BB** (0.073g) in 57% yield.

MH^+ 240.3, $MH+Na^+$ 486.4.

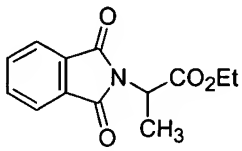
^{1}H NMR (CD_3OD, δ ppm) 1.2-2.0 (m, 6H), 2.35 (t, 2H), 2.55-2.82 (m, 4H), 3.68 (dt, 1H), 4.05 (t, 1H), 5.65 (m, 2H).

Example CC

(*E*)-2-amino-2-methyl-6-[(1-iminoethyl)amino]-4-hexenoic acid, dihydrochloride



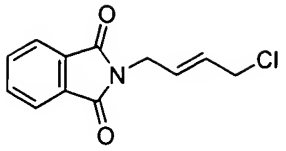
Example CC-1)



DL-Alanine ethyl ester hydrochloride (5 g, 32.5 mmol) was suspended in toluene (50 mL). Triethyl amine (4.5 mL, 32.5 mmol) was added followed by phthalic anhydride (4.8 g, 32.5 mL). The reaction flask was outfitted with a Dean-Stark trap and reflux condenser and the mixture was heated at reflux overnight. Approximately 10 mL of toluene / water was collected. The reaction mixture was cooled to room temperature and diluted with aqueous NH_4Cl and EtOAc. The layers were separated and the aqueous layer was extracted with EtOAc (3X). The ethyl acetate extract was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give the title phthalyl-protected amino ester as a white crystalline solid in near quantitative yield.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.2 (t, 3H), 1.6 (d, 3H), 4.2 (m, 2H), 4.9 (q, 1H), 7.7 (m, 2H), 7.9 (m, 2H)

Example CC-2)

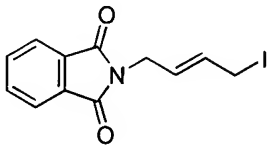


Potassium phthalimide (18.5g, 0.1 mol) was added to a 250 mL round bottomed flask containing 1,4-butene dichloride (25g, 0.2 mol). The reaction mixture was heated to 150 °C for 1.5 h. The mixture was cooled to room temperature and was partitioned between brine and Et_2O. The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuo. The residue was recrystallized from hot ethanol to give the title 1-chloro-4-phthalimidobutene (8.9g, 39%) as orange crystals.

HRMS calcd. For $C_{12}H_{10}ClNO_2$: *m/z* = 236.0478 [M+H]. Found: 236.0449

^1H NMR (300 MHz, $CDCl_3$, δ ppm: 4.1 (d, 2H), 4.3 (d, 2H), 5.9 (m, 2H), 7.7 (m, 2H), 7.9 (m, 2H)

Example CC-3)

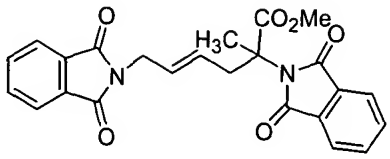


A sample of the product of **Example CC-2** (2.3g, 9.8 mmol) was dissolved in acetone (50 mL). NaI (3.2g, 21 mmol) was added and the mixture was refluxed overnight. After cooling to room temperature, Et_2O was added and the mixture was washed sequentially with sodium thiosulfate and brine. The organic layer was dried with $MgSO_4$, filtered and concentrated in vacuo to give the title iodide (2.8g, 87.5%) as a light yellow solid that was used without further purification.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 3.8 (d, 2H), 4.2 (d, 2H), 5.7 (m, 1H), 6.0 (m, 1H), 7.7 (m, 2H), 7.9 (m, 2H)

Mass (M+1)=328

Example CC-4)

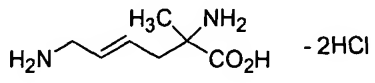


A solution of KHMDS (2.6 g, 13.3 mmol) in THF (50 mL) was cooled to -78 °C. A solution of the product of **Example CC-1** (2.2 g, 8.87 mmol) in THF (15 mL) was added and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU, 1.0 mL, 8.87 mL) was added immediately thereafter. After the solution was stirred at -78 °C for 40 minutes, a solution of the product of **Example CC-3** (2.9 g, 8 87 mmol) in THF (15 mL) was added. The flask was removed from the cold bath and was stirred at room temperature for 3h. The reaction mixture was partitioned between saturated aqueous $NaHCO_3$ and EtOAc. The organic extract was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo to give the desired bis-pththalyl protected amino ester as a yellow solid. This residue was chromatographed on silica gel (1:1 hexanes: EtOAc) and gave 1.4 g (35 %) of the title material as a white solid.

^{1}H NMR (300 MHz, $CDCl_3$, δ ppm: 1.2 (t, 3H), 1.6 (d, 3H), 2.8 (dd, 1H), 3.1 (dd, 1H), 4.2 (m, 4H), 5.6 (m, 1H), 5.8 (m, 1H), 7.6 (m, 4H), 7.7 (m, 2H), 7.9 (m, 2H)

Mass (M+H)=447

Example CC-5)



The product of **Example CC-4** (0.78 g, 1.76 mmol) was dissolved in a mixture of formic acid (10mL, 95%) and HCl (20 mL, concentrated HCl) and was refluxed for 3 days. The reaction mixture was cooled to 0 °C and filtered to remove phthalic anhydride. After concentrating in vacuo (T < 40 °C), the title unsaturated alpha methyl lysine was obtained as a white solid (0.38g, 95 %), which was used without further purification.

^{1}H NMR (300 MHz, D_2O, δ ppm): 1.4 (s, 3H), 2.4 (dd, 1H), 2.6 (dd, 1H), 3.5 (d, 2H), 5.7 (m, 2H)

Mass(M+H)=317

Example CC)

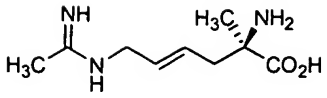
The product of **Example CC-5** (0.2 g, 0.86 mmol) was dissolved in H_2O (8 mL) and was brought to pH 9 with 2.5 N NaOH. Ethyl acetimidate - HCl (0.42 g, 3.4 mmol) was added in four portions over 1 h. After 1h, the mixture was acidified to pH 4 with 10% HCl and was concentrated in vacuo. The residue was then passed through a water-washed DOWEX 50WX4-200 column (H form, 0.5 N NH_4OH eluent). The residue was concentrated in vacuo, acidified to pH 4 with 10 % HCl, and concentrated to give the title product (17 mg, 6 %) as an oil.

HRMS calcd. For $C_9H_{17}N_3O_2$: *m/z* = 200.1399 [M+H]. Found: 200.1417

^{1}H NMR (400 MHz, D_2O, δ ppm): 1.4 (s, 3H), 2.1 (s, 3H), 2.5 (dd, 1H), 2.6 (dd, 1H), 3.8 (d, 2H), 5.6 (m, 2H)

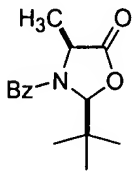
Example DD

(*R*, *E*)-2-amino-2-methyl-6-[(1-iminoethyl)amino]-4-hexenoic acid, dihydrochloride



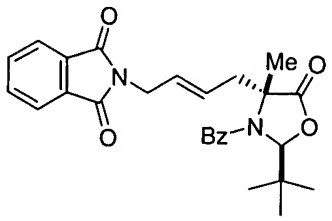
- 2HCl

Example DD-1)



(2*S*, 4*S*)- 3-Benzoyl-2-(tert-butyl)-4-methyl-1,3-oxazolidin-5-one was prepared according to Seebach's procedure. Seebach, D.; Fadel, A. Helvetica Chimica Acta 1985, 68, 1243.

Example DD-2)

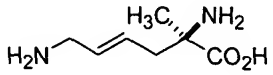


A solution of KHMDS (0.65g, 3.24 mmol), DMPU (0.33 mL, 2.7 mmol) and THF (40 mL) was cooled to -78 °C. A solution of (2*S*, 4*S*)- 3-benzoyl-2-(tert-butyl)-4-methyl-1,3-oxazolidin-5-one (**Example DD-1)** (0.70g, 2.7 mmol) in THF (10 mL) was added dropwise. After 45 min, a solution of the product of **Example CC-3** (0.88g, 2.7 mmol) in THF (10 mL) was added. The reaction mixture was stirred at room temperature for 2 h and quenched with saturated aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with EtOAc. The organic layers were combined and washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo. The resulting yellow oil was chromatographed on silica gel (9:1 then 4:1 hexanes / ethyl acetate) to give the title protected unsaturated alpha methyl D-lysine (0.26g, 20 %) as a colorless oil.

HRMS calcd. For $C_{27}H_{28}N_2O_5$: *m/z* = 461.2076[M+H]. Found: 461.2033

^{1}H NMR (400 MHz, $CDCl_3$,δ ppm: 0.9 (s, 9H), 1.5 (s, 3H), 4.3 (m, 2H), 5.5 (m, 2H), 5.6 (m, 2H), 6.1 (m, 1H), 7.5 (m, 5H), 7.7 (m, 2H), 7.9 (m, 2H)

Example DD-3)



- 2HCl

The product of **Example DD-2** (0.255 mg, 0.55 mmol) was dissolved in 6N HCl (6 mL) and formic acid (6 mL) and was heated to reflux for 24 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was suspended in water and washed with CH_2Cl_2. The aqueous layer was concentrated and passed through a water-washed DOWEX 50WX4-200 column (H form, 0.5 N NH_4OH eluent). The residue was concentrated in vacuo, acidified to pH 4 with 10 % HCl, and concentrated to give the title unsaturated D-lysine (71 mg, 55 %) as an oil which was used without further purification.

^{1}H NMR (400 MHz, D_2O, δ ppm:1.4 (s, 3H), 2.5 (dd, 1H), 2.6 (dd, 1H), 3.4 (d, 2H), 5.6 (m, 2H), 5.7 (m, 2H)

Example DD)

The product of **Example DD-3** (13 mg, 0.056 mmol) was dissolved in H_2O (5 mL) and was brought to pH 9 with 2.5 N NaOH. Ethyl acetimidate - HCl (27 mg, 0.2 mmol) was added in four portions over 2 h. After 2h, the mixture was acidified to pH 4 with 10% HCl and was concentrated in vacuo. The residue was passed through a water-washed DOWEX 50WX4-200 column (H form,

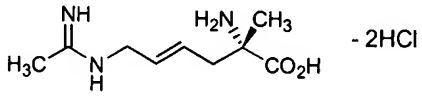
0.5 N NH_4OH eluent). The residue was concentrated in vacuo, acidified to pH 4 with 10 % HCl, and concentrated to give the title product (45 mg) as an oil.

HRMS calcd. For $C_9H_{17}N_3O_2$: *m/z* = 200.1399 [M+H]. Found: 200.1386

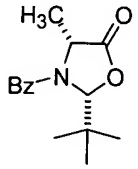
^{1}H NMR (400 MHz, D_2O, δ ppm): 1.4 (s, 3H), 2.1 (s, 3H), 2.5 (dd, 1H), 2.6 (dd, 1H), 3.8 (d, 2H), 5.6 (m, 2H)

Example E

(*S*, *E*)-2-amino-2-methyl-6-[(1-iminoethyl)amino]-4-hexenoic acid, dihydrochloride

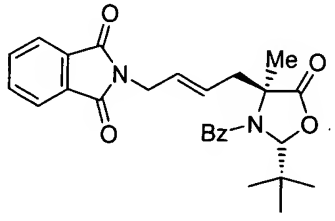


Example EE-1)



(2*R*, 4*R*)-3-Benzoyl-2-(tert-butyl)-4-methyl-1,3-oxazolidin-5-one was prepared according to Seebach's procedure. Seebach, D.; Fadel, A. Helvetica Chimica Acta 1985, 68, 1243.

Example EE-2)

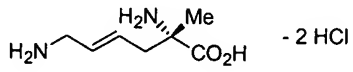


A solution of the (2*R*, 4*R*)-3-benzoyl-2-(tert-butyl)-4-methyl-1,3-oxazolidin-5-one product of **Example EE-1** (2.0g, 7.6 mmol) in THF (50 mL) was cooled to -78 °C. A -78 °C solution of KHMDS (0.65g, 3.24 mmol) in THF (25 mL) was added dropwise. After 30 min, a solution of the product of **Example CC-3** (2.8 g, 8.6 mmol) in THF (25 mL) was added. The reaction mixture was stirred at room temperature for 1 h and quenched with saturated aqueous $NaHCO_3$. The layers were separated and the aqueous layer was extracted with EtOAc. The organic layers were combined and washed with brine, dried with $MgSO_4$, filtered and concentrated in vacuo. The resulting orange oil was chromatographed on silica gel (9:1 then 4:1 hexanes / ethyl acetate) to give the protected title unsaturated alpha methyl L-lysine (0.5g, 15 %) as a white solid.

HRMS calcd. For $C_{27}H_{28}N_2O_5$: *m/z* = 461.2076[M+H]. Found: 461.2043

^{1}H NMR (400 MHz, $CDCl_3$, δppm): 0.9 (s, 9H), 1.5 (s, 3H), 4.3 (m, 2H), 5.5 (m, 2H), 5.6 (m, 2H), 6.1 (m, 1H), 7.5 (m, 5H), 7.7 (m, 2H), 7.9 (m, 2H)

Example EE-3)



The product of **Example EE-2** (0.5 g, 1 mmol) was dissolved in 12N HCl (10 mL) and formic acid (5 mL) and this mixture was heated to reflux for 12 h. The reaction mixture was cooled in the freezer for 3h and the solids were removed by filtration. The residue was washed with CH_2Cl_2 and EtOAc. The aqueous layer was concentrated in vacuo and gave the title unsaturated alpha methyl L-lysine (0.26 g, 99 %) as an oil which was used without further purification.

^{1}H NMR (300 MHz, D_2O,δ ppm): 1.4 (s, 3H), 2.5 (dd, 1H), 2.6 (dd, 1H), 3.4 (d, 2H), 5.7 (m, 2H)

Example EE)

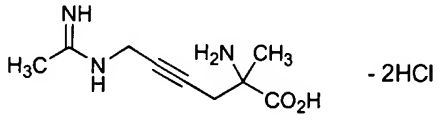
The product of **Example EE-3** (0.13 g, 0.56 mmol) was dissolved in H_2O (1 mL) and was brought to pH 9 with 2.5 N NaOH. Ethyl acetimidate - HCl (0.28 g, 2.2 mmol) was added in four portions over 1 h. After 1h, the mixture was acidified to pH 4 with 10% HCl and was concentrated in vacuo. The residue was and passed through a water-washed DOWEX 50WX4-200 column (0.5 N NH_4OH eluent). The residue was concentrated in vacuo, acidified to pH 4 with 10 % HCl, and concentrated to give the title product as an oil (40 mg).

HRMS calcd. For $C_9H_{17}N_3O_2$: *m/z* = 222.1218 [M+Na]. Found: 222.1213

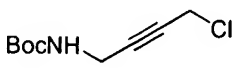
^{1}H NMR (300 MHz, D_2O, δ ppm): 1.4 (s, 3H), 2.1 (s, 3H), 2.4 (dd, 1H), 2.6 (dd, 1H), 3.8 (d, 2H), 5.6 (m, 2H)

Example FF

2-amino-2-methyl-6-[(1-iminoethyl)amino]-4-hexynoic acid, dihydrochloride

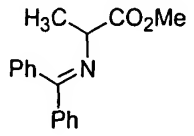


Example FF-1)



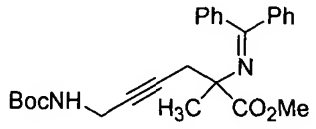
The N-boc-1-amino-4-chlorobut-2-yne was prepared following the procedure described in Tetrahedron Lett. 21, 4263 (1980).

Example FF-2)



Methyl N-(diphenylmethylene)-L-alaninate was prepared by following the procedure described in J. Org. Chem., 47, 2663 (1982).

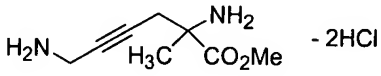
Example FF-3)



Dry THF (1000mL) was placed in a flask purged with argon and 60% NaH dispersed in mineral oil (9.04 g, 0.227 mol) was added. To this mixture was added the product of **Example FF-2** (30.7 g, 0.114 mol). The reaction mixture was then stirred at 10 °C - 15°C for 30 min. Potassium iodide (4 g) and iodine (2 g) were added and immediately followed by the addition of the product of **Example FF-2** (23 g, 0.113 mol in 200 mL THF) in 30 min. The reaction mixture was then stirred at 55 °C until the starting material disappeared (~ 2 h). The reaction mixture was then cooled to room temperature and the solvent was evaporated. Ethyl acetate (500 mL) was added and the mixture was carefully washed with 2 X 200 mL deionized water. The organic layer was dried over anhydrous $MgSO_4$, filtered and evaporated to give 44 g of crude product. Purification by chromatography using 20% ethyl acetate in hexane afforded the title protected unsaturated alpha-methyl lysine (28 g, 57%).

Anal.Calcd for $C_{26}H_{30}N_2O_4$ and 0.5 ethylacetate: C,70.42; H, 7.14; N, 5.91.
Found: C, 70.95; H, 7.73; N, 6.09
IR (Neat, λ max, cm^{-1}): 2981, 1714, 1631
^1H NMR ($CDCl_3$, δ ppm): 1.28 (s, 9H), 1.4 (s, 3H), 2.65-2.76(m, 2H), 3.15 (s, 3H), 3.7 (bs, 2H), 4.6 (bs, 1H), 6.95-7.4 (m, 10H)
^{13}C NMR ($CDCl_3$, δ ppm): 24.29, 28.33, 28.39, 33.24, 51.60, 53.55, 127.79, 127.97, 128.26, 128.36, 128.43, 128.54, 128.66, 130.05, 130.22, 132.39
Mass (M+1) = 435
DSC purity: 261.95 °C

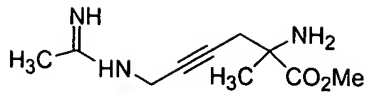
Example FF-4)



The product of **Example FF-3** (16 g, 0.0368 mol) was dissolved in 1N HCl (300 mL) and stirred at 25 °C for 2 h. The reaction mixture was washed with ether (2 x 150mL) and the aqueous layer separated and decolorized with charcoal. Concentration afforded ~9 g (100% yield) of the deprotected unsaturated alpha-methyl lysine ester **FF-4** as white foamy solid.

Anal.Calcd for $C_8H_{14}N_2O_2$ containing 2.26 HCl and 1.19 H_2O: C,35.06; H, 6.86; N, 10.22; Cl, 29.24. Found: C, 35.31; H, 7.38; N, 10.70; Cl, 29.77
^1H NMR (D_2O, δ ppm): 1.56 (s, 3H), 2.8-3.0 (2 dt, 2H), 3.75(s, 2H), 3.79 (s, 3H)
^{13}C NMR (D_2O, δ ppm): 23.89, 29.81, 32.05, 57.08, 61.90, 79.57, 82.43, 173.92
Mass (M+1) = 171
DSC purity: 114.22 °C
UV = 206 nm,abs 0.013
$[\alpha]_{25}$ in methanol = 0 at 365 nm

Example FF-5)



The product of **Example FF-4** (2.43 g, 0.01 mol) was dissolved in deionized water (25 mL). A solution of NaOH (400 mg, 0.01 mol) in deionized water (25 mL) was added at 25°C to bring the pH to ~7.95 and stirring was continued another 10 min. Ethylacetimidate hydrochloride (988 mg, 0.008 mol) was added to the reaction mixture with simultaneous adjustment of the pH to ~ 8.5 by adding 1N NaOH. The reaction mixture was stirred at pH 8 to 8.5 for 3 h following acetimidate addition. 1N HCl was added to the reaction mixture (4.1 pH). The solvent was evaporated at 50 °C to afford a yellow crude hygroscopic residue (4 g, >100% yield). Purification was carried out on the Gilson chromatography system using 0.1% AcOH/CH_3CN/H_2O.

Anal.Calcd for $C_{10}H_{17}N_3O_2$ containing 2.25 HCl and 1.7 H_2O: C, 37.08; H, 7.05; N, 12.97; Cl, 24.63. Found: C, 37.01; H, 6.79; N, 12.76; Cl, 24.87

IR (Neat, λ max, cm^{-1}): 2953, 2569, 1747, 1681, 1631

^1H NMR (D_2O, δ ppm): 1.52 (s, 3H), 2.12 (s, 3H), 2.74-2.96 (2 dt, 2H), 3.75 (s, 3H), 3.95 (t, 2H)

^{13}C NMR (D_2O, δ ppm): 23.89, 29.81, 32.05, 57.08, 61.90, 79.57, 82.43, 173.92

Mass (M+1) = 212

Example FF)

The product of **Example FF-5** (100 mg, 0.0005 mol) was dissolved in 8N HCl (20 mL) and stirred for 10 h at reflux. The reaction mixture was cooled to room temperature and the aq. HCl was evaporated on rotavap. The residue was dissolved in deionized water (10mL) and water and reconcentrated under

vacuum to afford the title product as a yellow glassy solid in almost quantitative yield (88 mg).

Anal.Calcd for $C_9H_{15}N_3O_2$ containing 2.4 HCl and 1.8 H_2O: C, 34.08; H, 6.67; N, 13.25; Cl, 26.83. Found: C, 34.32; H, 6.75; N, 13.63; Cl, 26.47

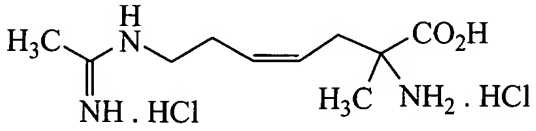
IR (Neat, λ max, cm^{-1}): 1738, 1677, 1628, 1587

^1H NMR (D_2O, δ ppm): 1.6 (s, 3H), 2.24 (s, 3H), 2.8-3.0 (2 dt, 2H), 4.1 (s, 2H)

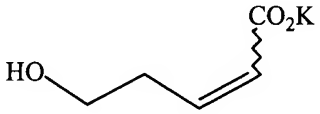
^{13}C NMR (D_2O, δ ppm): 21.22, 24.10, 29.88, 34.58, 80.04, 80.99, 128.39, 168.07, 176.13

Mass (M+1) = 198

Example GG



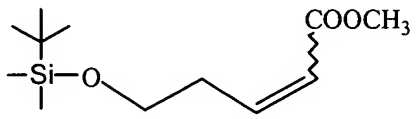
(2*R*/*S*,4*Z*)-2-amino-2-methyl-7-[(1-iminoethyl)amino]-4-heptenoic acid, dihydrochloride



Example GG-1) 5,6 dihydropyran-2-one (49.05g, 0.5mol) was dissolved in 200 mL of water. Potassium hydroxide (35g, 0.625 mol) was added and the reaction mixture stirred at ambient temperature for 5 hours. The solvent was removed in vacuo to yield a colorless glassy solid (65g, 84%) that was

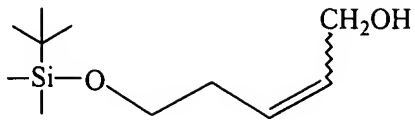
characterized by NMR to be predominantly the cis isomer of the title compound.

^{1}H NMR ($CDCl_3$) δ: 2.7 (m, 2H), 3.6 (t, 2H), 5.8-5.85(m, 1H), 5.9-5.97 (m, 1H).



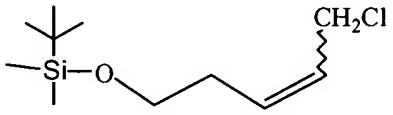
Example GG-2) The product of **Example GG-1** was dissolved in 100 mL of dimethyl formamide. Methyl Iodide (52mL, 0.84 mol) was then added resulting in an exotherm to 40 °C. The reaction mixture was stirred at room temperature for 10 hours and partitioned between 150 mL of ethylacetate / diethylether in a 20/ 80 ratio and ice water. The aqueous layer was separated and re-extracted with 100 mL of diethyl ether. The organic layers were combined , dried (Na_2SO_4), filtered and stripped of all solvent to yield the desired methyl ester product (40g, 71%). This material was dissolved in 200 mL of methylene chloride and the solution cooled to 0°C. Tertiarybutyl dimethylsilylchloride, triethylamine and dimethylaminopyridine were added. The reaction mixture was slowly warmed to room temperature and stirred for 10 hours under nitrogen. The reaction was extracted with 100 mL of 1N aqueous potassium bisulfate solution. The organic layer was washed with 2X 100 mL of brine and then with 3 X 150 mL of water. The organic layer was dried (Na_2SO_4), filtered and stripped to yield 42g (56%) of the title material.

^{1}H NMR ($CDCl_3$) δ: 0.02 (s, 6H), 0.085 (s, 9H), 2.8-2.85 (m, 2H), 3.65 (s, 3H), 3.66-3.7 (m 2H), 5.8 (m, 1H), 6.3 (m, 1H)



Example GG-3) The material from **Example GG-2** was dissolved in 25 mL of toluene and cooled to 0°C. Diisobutylaluminum hydride (1.0 M in toluene, 32 mL, 48 mmol) was added dropwise maintaining the temperature between 5 and -10 °C. The reaction mixture was stirred for 1.5 hours between 6 and -8 °C before it was cooled to -25 °C. To this mixture was added 100 mL of 0.5N sodium potassium tartarate. The reaction mixture was allowed to warm up to room temperature and stirr for an hour. A gelatinous precipitate was formed which was filtered. The aqueous was extracted with 2 X 100 mL EtOAc. The combined organic layers were dried (sodium sulfate), filtered and concentrated in vacuo to yield title product (3.45g, 66%) as a colorless oil.

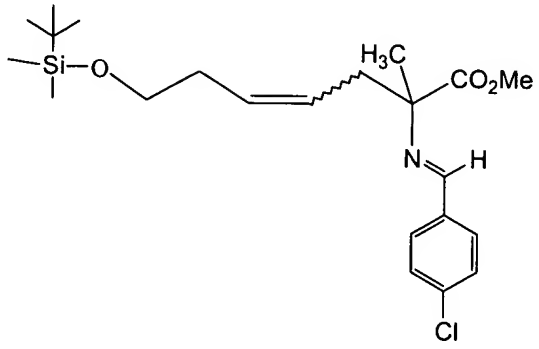
^{1}H NMR ($CDCl_3$) δ: 0.02 (s, 6H), 0.085 (s, 9H), 2.25-2.32 (m, 2H), 2.6 (bs, 1H), 3.6 (t, 2H), 4.08 (d, 2H), 5.45-5.55 (m, 1H), 5.7-5.75 (m, 1H)



Example GG-4) The product (8g, 37 mmol) from **Example GG-3** was dissolved in 100 mL methylene chloride and this solution was cooled to 0 °C. Methanesulfonyl chloride was then added and this mixture was stirred for 5 min. Triethylamine was then added. The temperature maintained between 0 and -10 °C during the addition of the aforementioned reagents. The reaction mixture was subsequently warmed up to room temperature and stirred for 24 hours. It was then extracted with 100 mL of 50% aqueous sodium bicarbonate solution. The organic layer was washed with 100 mL of saturated aqueous

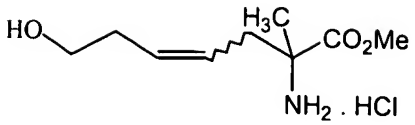
brine solution, dried (sodium sulfate), filtered and stripped in vacuo to yield the title material (8.2g, 94%).

^{1}H NMR ($CDCl_3$) δ: 0.02 (s, 6H), 0.085 (s, 9H), 2.25-2.32 (m, 2H), 3.6 (t, 2H), 4.08 (d, 2H), 5.6-5.7 (m, 2H)



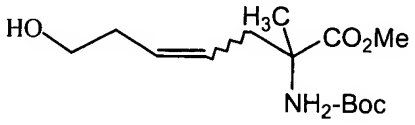
Example GG-5) A solution of N-p-chloro phenylimine alanine methyl ester (8.85g, 34 mmol) dissolved in 59 mL of tetrahydrofuran was purged with Argon. NaH (1.64g, 41mmol) was added whereupon the solution turned bright orange and subsequently a deep red. A solution of the title material from **Example GG-4** (8g, 34 mmol) in 40 mL of tetrahydrofuran was added to the above anionic solution. An exotherm was observed raising the temperature to almost 40°C. The reaction mixture was maintained between 48 and -52 °C for 2 hours. It was then cooled to room temperature and filtered. Filtrate was stripped in vacuo to yield the title material (8.4g, 50% crude yield) as a yellow oil.

^{1}H NMR ($CDCl_3$) δ: 0.02 (s, 6H), 0.085 (s, 9H), 1.45 (s, 3H), 1.6 (s, 1H), 2.2-2.25(m, 2H), 2.65 (d, 2H), 3.55 (m, 2H), 3.7 (s, 3H), 5.45-5.55 (m, 2H), 7.35-7.7 (m, 4H)



Example GG-6) The title material from **Example GG-5** (8.4g, 18.2mmol) was treated with 125 mL 1N hydrochloric acid and the reaction was stirred for an hour at room temperature. After the reaction mixture had been extracted 2 X 75 mL of ethylacetate the aqueous layer was stripped in vacuo at 56°C to yield 4g of the title material (100% crude yield).

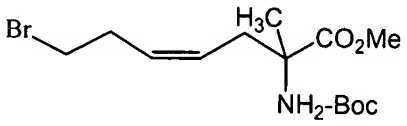
^{1}H NMR (CD_3OD) δ: 1.6 (s, 3H), 2.3-2.4 (m, 2H), 2.65-2.8 (m, 2H), 3.6-3.65 (m, 2H), 3.87 (s, 3H), 5.4-5.5 (m, 1H), 5.75-5.85 (m, 1H)



Example GG-7) The title product of **Example GG-6** (1.9g, 8.5 mmol) was dissolved in a mixture of 15mL dioxane and 8mL of water. Solid potassium bicarbonate was then carefully added to avoid foaming. The reaction mixture was stirred for 10 min before tertiarybutyloxycarbonyl anhydride was added portion-wise and reaction mixture was stirred at ambient temperature for 24 hours. The reaction mixture was diluted with 100 mL of ethylacetate and 50 mL of water before it was poured into a separatory funnel. The organic layer was separated, dried (Na_2SO_4), filtered and stripped to yield the title material as a colorless oil (1.9g, 78% crude yield).

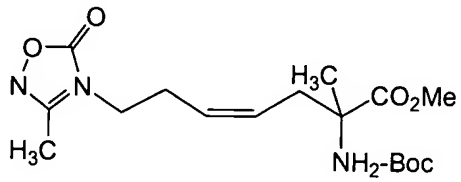
^{1}H NMR ($CDCl_3$) δ: 1.42 (s, 9H), 1.55 (s, 3H), 2.3-2.36 (m, 2H), 2.58-2.65 (m, 2H), 3.65-3.7 (t, 2H), 3.75 (s, 3H), 5.42-5.5 (m, 1H), 5.55-5.62 (m, 1H)

Example GG-8) Another 1.9 g sample of the title material from **Example GG-6** was converted by the methods of **Example GG-7** to the crude Z / E mixture of the title product of **Example GG-7**. This material further purified on silica with a solvent system of ethylacetate / hexane in a 20/80 ratio to obtain the minor E-isomer as well as the major Z-isomer.



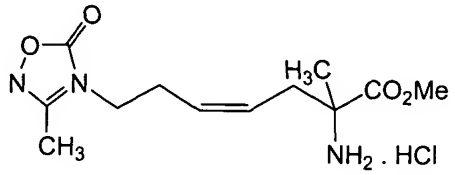
Example GG-9) The title Z-isomer from **Example GG-8** (1.8 g, 6.25 mmol) was dissolved in 20mL of acetonitrile and this solution was cooled to 0 °C. Pyridine (0.76g, 9.4mmol) was then added followed by the portion-wise addition of solid dibromotriphenylphosphorane (3.46g, 8.2mmol) over 10 min. The reaction mixture was stirred under Argon for 24 hours at room temperature. The precipitate that formed was filtered off. The filtrate was concentrated in vacuo to give 2.8 g of an oil that was purified on silica gel using a solvent system of ethylacetate / hexane in a 60/ 40 ratio. The 1.1g of title material (50 %) was characterized by NMR.

^{1}H NMR ($CDCl_3$) δ: 1.44 (s, 9H), 1.55 (s, 3H), 2.6-2.65 (m, 4H), 3.35-3.4 (m, 2H), 3.75 (s, 3H), 5.4-5.45 (m, 1H), 5.55-5.6 (m, 1H)



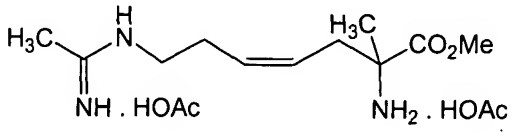
Example GG-10) The title material from **Example GG-8** (300mg, 0.86mmol) was dissolved in 25 mL of dimethylformamide (DMF). The potassium salt of 3-methyl-1,2,4-oxadiazolin-5-one (130mg, 0.94mmol) was added and the reaction mixture was heated to 52°C and maintained there for 18 hours with stirring. It was then cooled to room temperature before the DMF was stripped in vacuo at 60°C. The residue was purified on silica gel with a gradient of 60/40 to 90/10 ethyl acetate/ hexane to yield 300 mg (95 %) of the title material.

^{1}H NMR (CD_3OD) δ: 1.35 (s, 3H), 1.43 (s, 9H), 2.32 (s, 3H), 2.45-2.55 (m, 4H), 3.65-3.7 (m, 2H), 3.72 (t, 3H), 5.5-5.6 (m, 2H)



Example GG-11) The product of **Example GG-10** (300mg) was treated with 0.05 N of aqueous HCl and this solution was stirred for 30 min. The solvent was removed in vacuo to afford the desired material in nearly quantitative yield.

^{1}H NMR (CD_3OD) δ: 1.6 (s, 3H), 2.25 (s, 3H), 2.45-2.55 (m, 2H), 2.7-2.8 (m, 2H), 3.3-3.4(m, 5H), 5.5-5.6 (m, 1H), 5.7-5.8 (m, 1H)



Example GG-12) The title material from **Example GG-11** (198 mg, 0.54 mmol) was dissolved in 50 mL of MeOH. Formic acid (40mg) was then added followed by Palladium on Calcium carbonate (400 mg). The reaction mixture was heated to 65 °C with stirring in a sealed tube for 24 hours. It was then cooled to room temperature and filtered. The filtrate was concentrated in vacuo and the residue purified by reverse phase HPLC to yield 115 mg (75%) of the title material.

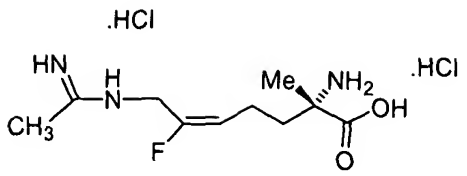
^{1}H NMR (CD_3OD) δ: 1.4 (s, 3H), 1.95 (s, 3H), 2.25 (s, 3H), 2.4-2.52 (m, 4H), 3.25-3.35 (m, 2H), 3.75 (t, 3H), 5.54-5.62 (m, 2H)

Example GG) The title material (75 mg) from **Example GG-12** was dissolved in 15 mL of 2N hydrochloric acid. The reaction mixture was heated to a reflux and stirred for 6 hours before ot was cooled to room temperature. The solvent was removed in vacuo. The residue was dissolved in 25 mL of water and stripped on the rotary evaporator to remove excess hydrochloric acid. The residue was dissolved in water and lyophilized to give 76 mg (~100 %) of the title material.

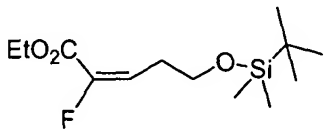
Elemental analyses Calcd for $C_{10}H_{19}N_3O_2$ + 2.2HCl + 2.2 H_2O: C, 36.06; H, 7.75; N, 12.61. Found for $C_{10}H_{19}N_3O_2$ + 2.2HCl + 2.2 H_2O: C, 35.91; H, 7.61; N, 12.31

^{1}H NMR (CD_3OD) δ: 1.47 (s, 3H), 2.32 (s, 3H), 2.45-2.64 (m, 4H), 2.58-2.65 (m, 2H), 3.65-3.7 (t, 2H), 5.55-5.65 (m, 2H)

Example HH



(2*S*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride

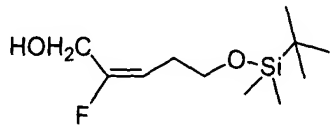


Example-HH-1) To a cold (-78 °C) solution of triethyl 2-fluorophosphonoacetate (25.4 g, 105 mmol) in 100 mL of THF was added *n*-butyl lithium (63 mL of 1.6 M in hexane, 101 mmol). This mixture was stirred at -78 °C for 20 min producing a bright yellow solution. A solution of crude 3-[(*tert*-butyldimethylsilyl)oxy]propanal (*J. Org. Chem.*, **1994**, *59*, 1139-1148) (20.0 g, 105 mmol) in 120 mL of THF was then added dropwise over ten minutes, and the resulting mixture was stirred for 1.5 h at -78 °C, at which time analysis by thin layer chromatography (5% ethyl acetate in hexane) showed that no starting material remained. The reaction was quenched at -78 °C with sat. aqueous NH_4Cl (150 mL). The organic layer was collected, and the aqueous layer was extracted with diethyl ether (300 mL). The combined organics were washed with brine (200 mL), dried over $MgSO_4$, filtered and concentrated. The crude material was filtered through a plug of silica gel (150 g) eluting with hexane (2 L) to give 14.38 g (52%) of the desired (2E)-5-[[(1,1-dimethylethyl)di-methylsilyl]oxy]-2-fluoro-2-pentenoic acid ethyl ester product as a clear oil. ^1H NMR and ^{19}F NMR indicated that the isolated product had an approximate E:Z ratio of 95:5.

HRMS calcd. for $C_{13}H_{26}FO_3Si$: *m/z* = 277.1635 $[M+H]^+$, found: 277.1645.

^1H NMR ($CDCl_3$) δ 0.06 (s, 6H), 0.94 (s, 9H), 1.38 (t, 3H), 2.74 (m, 2H), 3.70 (m, 2H), 4.31 (q, 2H), 6.0 (dt, vinyl, 1H).

^{19}F NMR ($CDCl_3$) δ -129.78 (d, 0.05 F, *J* = 35 Hz, 5% Z-isomer), -121.65 (d, 0.95 F, *J* = 23 Hz, 95% E-isomer).

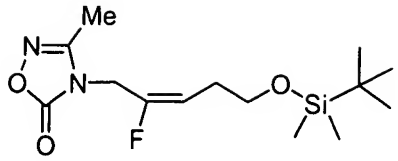


Example-HH-2) To a solution of **Example-HH-1** (6.76 g, 24.5 mmol) in 100 mL of methanol at room temperature was added solid $NaBH_4$ (4.2 g, 220 mmol) in 1.4 g portions over three hours. After 3.5 hours water was added (10 mL). Additional solid $NaBH_4$ (4.2 g, 220 mmol) was added in 1.4 g portions over three hours. The reaction was quenched with 150 mL of sat. aqueous NH_4Cl and extracted with diethyl ether (2 x 250 mL). The organic layers were combined, dried over $MgSO_4$, filtered and concentrated. The crude material, 4.81 g of clear oil, was purified by flash column chromatography on silica gel eluting with 10% ethyl acetate in hexane to give 2.39 g (42%) of the desired (2E)-5-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2-fluoro-2-penten-1-ol product as a clear oil, that contained an approximate E:Z ratio of 93:7 by ^{19}F NMR.

HRMS calcd. for $C_{11}H_{24}FO_2Si$: *m/z* = 235.1530 $[M+H]^+$, found: 235.1536.

^1H NMR ($CDCl_3$) δ 0.06 (s, 6H), 0.88 (s, 9H), 2.35 (m, 2H), 3.62 (t, 2H), 4.19 (dd, 2H), 5.2 (dt, vinyl, 1H).

^{19}F NMR ($CDCl_3$) δ -120.0 (dt, 0.07F, 7% Z-isomer), -109.82 (q, 0.93 F, *J* = 21 Hz, 93% E-isomer).

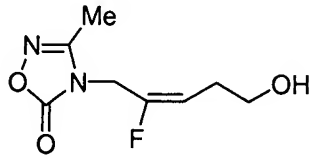


Example-HH-3) To a mixture of **Example-HH-2** (2.25 g, 9.58 mmol), polymer-supported triphenylphosphine (3 mmol/g, 1.86 g, 15 mmol) and 3-methyl-1,2,4-oxadiazolin-5-one (1.25 g, 12.5 mmol) in 60 mL of THF was added dropwise diethylazodicarboxylate (2.35 mL, 14.7 mmol). The reaction mixture was stirred for 1 h at room temperature, and additional 3-methyl-1,2,4-oxadiazolin-5-one (0.30 g, 3.0 mmol) was added. After 30 minutes, the mixture was filtered through celite, and the filtrate was concentrated. The resulting yellow oil was triturated with diethyl ether (30 mL) and the solid removed by filtration. The filtrate was concentrated, triturated with hexane (30 mL) and filtered. The filtrates was concentrated to an oil which was purified by flash column chromatography on silica gel eluting with 15% ethyl acetate in hexane to give 1.83 g (60%) of the desired 4-[(2E)-5-[[(1,1-dimethylethyl)dimethylsilyl]oxy]-2-fluoro-2-pentenyl]-3-methyl-1,2,4-oxadi-azol-5(4H)-one product as a clear oil, that contained only the desired E-isomer by ^{19}F NMR.

HRMS calcd. for $C_{14}H_{26}FN_2O_3Si$: *m/z* = 317.1697 $[M+H]^+$, found: 317.1699.

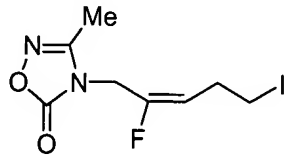
^{1}H NMR ($CDCl_3$) δ 0.04 (s, 6H), 0.85 (s, 9H), 2.28 (s, 3H), 2.37 (m, 2H), 3.64 (t, 2H), 4.32 (d, 2H), 5.4 (dt, vinyl, 1H).

^{19}F NMR ($CDCl_3$) δ -110.20 (q, 1 F, *J* = 21 Hz).



Example-HH-4) A solution of **Example-HH-3** (1.83 g, 5.78 mmol) in a mixture of acetic acid (6 mL), THF (2 mL) and water (2 mL) was stirred at room temperature for 2.5 hours. The resulting solution was concentrated *in vacuo* to an oil which was dissolved in diethyl ether (50 mL). The organic layer was washed with saturated $NaHCO_3$, and the aqueous layer was extracted with diethyl ether (2 x 50 mL) and ethyl acetate (2 x 50 mL). The combined organic layers were dried ($MgSO_4$), filtered and evaporated to give 1.15 g (98%) of the desired 4-[(2E)-2-fluoro-5-hydroxy-2-pentenyl]-3-methyl-1,2,4-oxadiazol-5(4H)-one product as a clear colorless oil.

HRMS calcd. for $C_8H_{12}FN_2O_3$: *m/z* = 203.0832 $[M+H]^+$, found: 203.0822. ^1H NMR ($CDCl_3$) δ 2.31 (3H), 2.4 (m, 2H), 3.66 (t, 2H), 4.37 (d, 2H), 5.42 (dt, vinyl, 1H). ^{19}F NMR ($CDCl_3$) δ -110.20 (q, 1 F, *J* = 21 Hz).

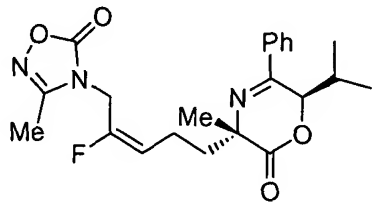


Example-HH-5) To a CH_2Cl_2 (2 mL) solution of triphenylphosphine (238 mg, 0.91 mmol) and imidazole (92 mg) at 0 °C was added solid iodine (230 mg, 0.91 mmol), and the mixture was stirred for 5 minutes. To the resulting yellow slurry was added a CH_2Cl_2 (1.5 mL) solution of **Example-HH-4** (0.15 g, 0.74 mmol). The slurry was allowed to warm to room temperature and stirred 30 minutes. The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with saturated $Na_2S_2O_3$ (5 mL) and brine (5 mL), dried ($MgSO_4$), filtered and evaporated to an oil. Addition of diethyl ether (10 mL) to the oil gave a white precipitate that was removed by filtration and the filtrate was concentrated to an oil. The crude material was purified by flash column chromatography on

silica gel eluting with 30% ethyl acetate in hexane to give 0.18 g (78%) of the desired 4-[(2E)-2-fluoro-5-iodo-2-pentenyl]-3-methyl-1,2,4-oxadiazol-5(4H)-one product as a clear oil, which solidified upon standing, mp = 58.1-58.6 °C.

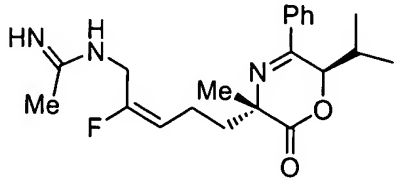
Anal. calcd. for $C_8H_{10}FIN_2O_2$: C, 30.79; H, 3.23; N, 8.98. Found: C, 30.83; H, 3.11; N, 8.85. HRMS calcd. for $C_8H_{11}FIN_2O_2$: *m/z* = 330.0115 $[M+H]^+$, found: 330.0104.

^{1}H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.75 (q, 2H), 3.21 (t, 2H), 4.31 (d, 2H), 5.39 (dt, vinyl, 1H). ^{19}F NMR ($CDCl_3$) δ -108.21 (q, 1F, *J* = 21 Hz).



Example-HH-6) To a 1-methyl-2-pyrrolidinone (12 mL) solution of (3*S*, 6*R*)-6-isopropyl-3-methyl-5-phenyl-3,6-dihydro-2*H*-1,4-oxazin-2-one (*Synthesis*, **1999**, *4*, 704-717) (1.10 g, 4.76 mmol), LiI (0.63 g, 4.76 mmol) and **Example-HH-5** (0.85 g, 2.72 mmol) in an ice bath was added 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (1.38 mL, 4.76 mmol). The yellow solution became orange upon addition of the base, and the resulting solution was allowed to stir at room temperature for 1 hour. The reaction mixture was diluted with ethyl acetate (100 mL), washed with water (2 x 30 mL), dried ($MgSO_4$), filtered and evaporated to a yellow oil. The crude material was purified by flash column chromatography on silica gel eluting with 30% ethyl acetate in hexane to give 0.64 g (57%) of the desired alkylated product as a clear oil.

^{1}H NMR (C_6D_6) δ 0.57 (d, 3H), 0.89 (d, 3H), 1.30 (s, 3H), 1.65 (s, 3H), 1.8 (m, 2H), 2.0 (m, 2H), 2.1 (m, 1H), 3.22 (m, 2H), 4.88 (dt, vinyl, 1H), 5.49 (d, 1H), 7.1 (m, 3H), 7.6 (m, 2H). ^{19}F NMR ($CDCl_3$) δ -110.37 (q, 1 F, J = 21 Hz).



Example-HH-7) To a methanol (20 mL) solution of **Example-HH-6** (0.13 g, 0.31 mmol) was added Lindlar catalyst (1.0 g). The stirred slurry was heated to 60 °C for 1 hour, and additional Lindlar catalyst (0.30 g) was added. The slurry was stirred an additional 1 hour at 60 °C, then cooled to room temperature. The catalyst was removed by filtration through celite, and the filtrate was stripped to give 0.58 g (100%) of the desired deprotected amidine product as a pale yellow oil.

MS: m/z = 374.2 $[M+H]^+$

^{1}H NMR (CD_3OD) δ 0.77 (d, 3H), 1.07 (d, 3H), 1.58 (s, 3H), 2.02 (s, 3H), 1.8-2.2 (m, 5H), 3.83 (d, 2H), 5.20 (dt, vinyl, 1H), 5.69 (d, 1H), 7.4 (m, 3H), 7.7 m, 2H)

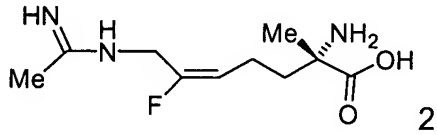
^{19}F NMR ($CDCl_3$) δ -109.4 (m, 1F, J = 21 Hz)

Example-HH) A solution of the product from **Example-HH-7** (0.58 g, 1.54 mmol) in 1.5 N HCl (25 mL) was washed with diethyl ether (2 x 20 mL) and refluxed for 1 hour. The solvent was stripped and the crude amino acid ester was dissolved in 6 N HCl (15 mL) and heated to reflux. After six hours, the solvent was removed *in vacuo*, and the resulting foam was purified by reverse-phase HPLC eluting with a 30 minute gradient of 0-40% CH_3CN/H_2O(0.25%

acetic acid). Fractions containing product were combined and concentrated to a foam. The product was dissolved in 1 N HCl and the solvent removed *in vacuo* (2x) to give 0.15 g (29%) of the desired (2*S*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product.

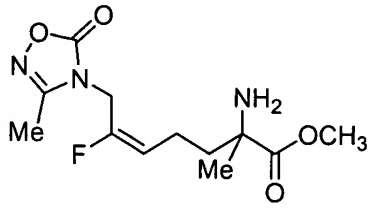
HRMS calcd. for $C_{10}H_{19}FN_3O_2$: *m/z* = 232.1461 $[M+H]^+$, found: 232.1485. ^1H NMR (D_2O) δ 1.43 (s, 3H), 2.10 (s, 3H), 1.8-2.1 (m, 4H), 3.98 (d, 2H) 5.29 (dt, vinyl, 1H). ^{19}F NMR ($CDCl_3$) δ -109.97 (q, 1 F, *J* = 21 Hz).

Example II



2HCl

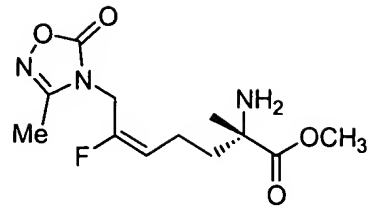
(2*S*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride



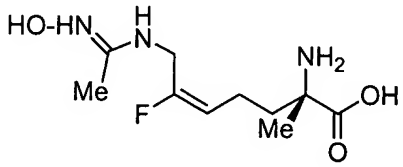
Example-II-1) To a 1-methyl-2-pyrrolidinone (7500 mL) solution of methyl N-[(3,4-dichlorophenyl)-methylene]-alaninate (748.5 g, 2.88 mol) under nitrogen was added LiI (385.5 g, 2.88 mol) and the resulting slurry stirred approximately 20 minutes to give a clear solution. The solid from **Example-HH-5** (750 g, 2.40 mol) was then added and the resulting solution cooled in an ice bath to ~0 °C. Neat BTPP (900 g, 2.88 mol) was added dropwise over 25 minutes

maintaining the internal temperature below 5 °C. After stirring for an additional 1.5 hour at 5 °C, the reaction was determined to be complete by HPLC. At this time, 7500 mL of methyl t-butyl ether (MTBE) was added followed by addition of 9750 mL of a water/crushed ice mixture. The temperature rose to 20 °C during this operation. After stirring vigorously for 5-10 minutes, the layers were separated and the aqueous layer washed with twice with 6000 mL of MTBE. The MTBE layers were combined and washed two times with 7500 mL of water. The resulting MTBE solution was then concentrated to ~5000 mL, treated with 11625 mL of 1.0 N HCl, and stirred vigorously at room temperature for one hour. The layers were separated and the aqueous layer washed with 7500 ml of MTBE. About 1 kg of sodium chloride was added to the aqueous layer and the resulting mixture stirred until all the salt had dissolved. At this point, 7500 mL of ethyl acetate was added, the resulting mixture cooled to 10° C, and 2025 mL of 6.0 N sodium hydroxide added with good agitation. The resulting pH should be about 9. The layers were separated and the aqueous layer was saturated with sodium chloride and extracted again with 7500 mL of ethyl acetate. The combined ethyl acetate extracts were dried ($MgSO_4$) and concentrated to a light oil. It should be noted that the ethyl acetate was not complete removed. With agitation, 3000 ml of hexane then is added to generate a slurry that was cooled to 10 °C. The granular solid was collected by filtration and washed with 1500 mL of hexane. About 564 g (82% yield) of the desired pure aminoester (>95% pure by HPLC) was obtained as a white solid, m.p. 82.9-83.0 °C. LCMS: *m/z* = 288.2 $[M+H]^+$. Chiral HPLC (Chiralpak-AD normal phase column, 100% acetonitrile, 210 nm, 1 mL/min): Two major peaks at 4.71 and 5.36 min (1:1).

^1H NMR ($CDCl_3$): δ 1.40 (s, 3H), 1.7-1.8 (m, 2H), 2.0 (br s, 2H), 2.2 (m, 2H), 2.29 (s, 3H), 3.73 (s, 3H), 4.34 (dd, 2H), 5.33 (dt, 1H).



Example-II-2) Separation of the individual enantiomers of the product from **Example-II-1** was accomplished on preparative scale using chiral HPLC chromatography (ChiralPak-AD, normal phase column, 100% acetonitrile) to give the desired pure (2S)-2-methyl amino ester product title product. ChiralPak-AD, normal phase column, 100% acetonitrile, 210 nm, 1 mL/min): 5.14 min (99%).



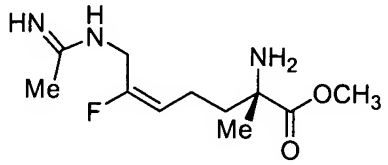
Example-II-3) A slurry of the product of **Example-II-2** (2.30 g, 8.01 mmol) in 0.993 M NaOH (30.0 ml, 29.79 mmol) was stirred 2 hours at room temperature. To the resulting clear colorless solution was added 1.023 M HCl (29.10 mL, 29.76 mmol). The resulting clear solution was concentrated until a precipitate began to form (approx. 30 mL). The slurry was warmed to give a clear solution that was allowed to stand at room temperature overnight. The precipitate was isolated by filtration. The solid was washed with cold water (2x10 mL), cold methanol (2x10 mL) and Et_2O (2x20 mL). The white solid was dried *in vacuo* at 40 °C 4 hours to give 1.04 g (53 %) of the desired N-hydroxy illustrated product. mp = 247.2 °C.

Anal. calcd. for $C_{10}H_{18}FN_3O_3$: C, 48.57; H, 7.34; N, 16.99; Cl, 0.0. Found: C, 48.49; H, 7.37; N, 16.91; Cl, 0.0.

HRMS calcd. for $C_{10}H_{19}FN_3O_3$: *m/z* = 248.1410 $[M+H]^+$, found: 248.1390.

^1H NMR (D_2O) δ 1.35 (s, 3H), 1.81 (s, 3H), 1.7-2.0 (m, 4H), 3.87 (d, 2H) 5.29 (dt, vinyl, 1H). ^{19}F NMR ($CDCl_3$) δ -112.51 (θ, 1 F, *J* = 21 Hz).

Example-II-4) To a solution of **Example-II-3** in methanol is added Lindlar catalyst. The stirred slurry is refluxed for 2 hours, then cooled to room temperature. The catalyst is removed by filtration through celite, and the filtrate is stripped. The resulting solid is dissolved in water and concentrated repeatedly from 1.0 N HCl to give the desired (2*R*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product.



Example-II-5) A solution of 73.5 g (0.3 mol) of the product from **Example-II-2** was dissolved in 300 mL of methanol and added dropwise to a preformed mixture of 13.7 g of Lindlar catalyst and 73.5 g of formic acid (1.53 mol) in 312 mL of methanol while maintaining the reaction temperature between 22 °C and 26 °C. After stirring at room temperature for an additional ~15 hrs, the reaction was determined to be complete by F^{19} NMR. The resulting reaction mixture was filtered through celite and the celite washed 3 times with 125 mL of methanol. The methanol filtrates were combined and concentrated to generate 115 g of the desired amidine title product as a viscous oil.

MS: m/z = 246 $(M+H)^+$.

^{1}H NMR (CD_3OD) δ1.6 (σ, 3H) 2.0–2.2 (m, 4H) 2.3 (s, 3H), 3.9 (s, 3H), 4.2 (d, 2H), 5.4 (dt,vinyl), 8.4 (s, 3H).

F^{19} NMR (CD_3OD) δ –110.4 (θ, J= 21 Hz) –111.7 (q, J=21 Hz).

In order to remove trace levels of lead, the crude product was dissolved in 750 mL of methanol and 150 g of a thiol-based resin (Deloxan THP 11) was added. After stirring 3 hrs at room temperature, the resin was filtered off and washed 2 times with 500 mL methanol. The filtrates were collected and concentrated to 99 g of the desired amidine title product as a viscous oil.

Alternatively:

A total of 5.0 g of the product from **Example-II-2** (0.0174 mole, 1.0 equiv) was mixed with 5.0 g of zinc dust (0.0765 moles, 4.39 equiv) in 40 mL of 1-butanol and 10 mL of acetic acid. After stirring for 5 hrs at 50 °C, LC analyses indicated the reaction to be complete. The solids were readily filtered off. The filtrate, after cooling in ice water to 7 °C, was treated with 30 mL of 6 N NaOH (0.180 moles) in one portion with vigorous stirring. After cooling the reaction mixture from 33 °C to 20 °C, the clear butanol layer was separated off and the aqueous layer extracted again with 40 mL of 1-butanol. The butanol extracts were combined, washed with 30 mL of brine followed by approx 10 mL of 6N HCl. After concentration at 70 °C, a clear glass resulted which was identified as the desired amidine title product.

Example-II) A solution of 99 g of the product from **Example-II-5** in 6 N HCl was refluxed for 1 hr at which time LC analyses indicated the reaction to be complete. The solvent was removed *in vacuo* to yield 89.2 g of a glassy oil which was dissolved in a mixture of 1466 mL ethanol and 7.5 ml of deionized water. THF was added to this agitated solution at ambient temperature until

the cloud point was reached (5.5 liters). An additional 30 ml of deionized water was added and the solution agitated overnight at room temperature. The resulting slurry was filtered and washed with 200 mL of THF to yield 65 g of a white solid identified as the desired title product.

$[\alpha]_D^{25}$ = +7.2 (c=0.9, H_2O)

mp = 126-130° C.

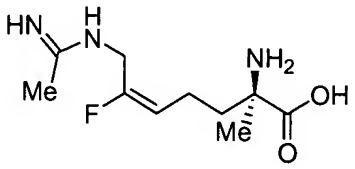
MS: m/z = 232 $(M+H)^+$.

Anal. Calcd for $C_{10}H_{22}N_3F_1O_3Cl_2$: C, 37.28; H, 6.88; N, 13.04; Cl, 22.01. Found: C, 37.52, H, 6.84, N, 13.21, Cl, 21.81.

^{1}H NMR (D_2O) δ 1.4 (σ, 3H), 1.8-2.1 (m, 4H), 1.9 (s,3H), 4.0(d, 2H), 5.3(dt, vinyl, 1H).

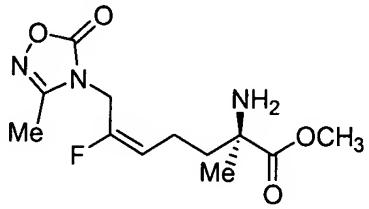
F^{19} NMR (D_2O) δ −109.6 (θ, J=21 Hz) −112.1 (q, J- 21 Hz).

Example JJ

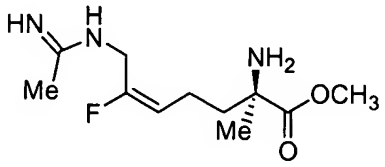


2HCl

(2*R*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride



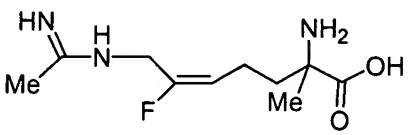
Example-JJ-1) Separation of the individual enantiomers of the product from **Example-II-1** was accomplished on preparative scale using chiral HPLC chromatography to give the desired pure (*2R*)-2-methyl amino ester product.



Example-JJ-2) The product from **Example-JJ-1** is dissolved in water and acetic acid. Zinc dust is added, and the mixture is heated at 60 °C until HPLC analysis shows that little of the starting material remains. The Zn is filtered through celite from the reaction mixture, and the filtrate is concentrated. The crude material is purified by reverse-phase HPLC column chromatography. Fractions containing product are combined and concentrated affording the desired (*2R*)-2-methyl acetamidine product.

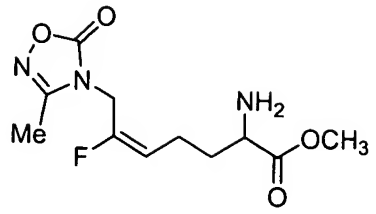
Example-JJ) A solution of **Example-JJ-2** in 2.0 N HCl is refluxed for 2 h. The solvent is removed *in vacuo*. The resulting solid is dissolved in water and concentrated repeatedly from 1.0 N HCl to give the desired (2*R*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product.

Example KK



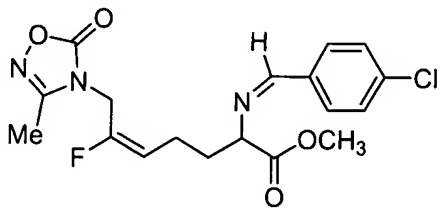
2HCl

(2*R/S*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride



Example-KK-1) To an 1-methyl-2-pyrrolidinone (5 mL) solution of methyl N-[(4-chlorophenyl)methylene]-glycinate (0.33 g, 1.6 mmol), LiI (0.20 g, 1.0 mmol) and a sample of the product of **Example-HH-5** (0.30 g, 0.96 mmol) in an ice bath was added 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (0.433 mL, 1.5 mmol). The solution was allowed to stir at room temperature for 1.5 hours. The reaction mixture was diluted with ethyl acetate (30 mL), washed with water (2 x 20 mL), dried ($MgSO_4$), filtered, and evaporated to give the crude desired racemic alkylated imine as a yellow oil.

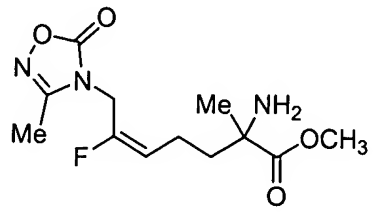
The crude material was dissolved in ethyl acetate (10 mL) and 1N HCl (10 mL) was added. The mixture was stirred for 2 hours at room temperature, and the organic layer was separated. The aqueous layer was neutralized with solid $NaHCO_3$ and extracted with ethyl acetate (2 x 30 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to give 0.13 g of the desired title racemic amino ester product as a yellow oil. This product was used in the next step without further purification. LCMS: *m/z* = 288.2 $[M+H]^+$.



Example-KK-2) To a CH_2Cl_2 (15 mL) solution of **Example-KK-1** (1.36 g, 4.98 mmol) was added 4-chlorobenzaldehyde (0.70 g, 5.0 mmol) and $MgSO_4$ (~5 g).

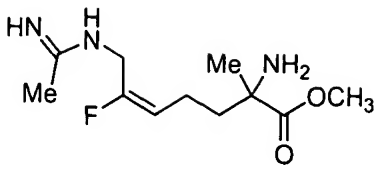
The slurry was stirred at room temperature for 18 hours. The slurry was filtered, and the filtrate stripped to give 1.98 g (100 %) of the desired title imine product as a pale yellow oil. This product was used in the next step without further purification.

^{1}H NMR (C_6D_6) δ 1.34 (s, 3H), 2.0 (br m, 4H), 3.32 (s, 3H), 3.42 (m, 2H), 3.83 (t, 1H), 4.98 (dt, vinyl, 1H).



Example-KK-3) To a CH_2Cl_2 (2 mL) solution of the product of **Example-KK-2** (0.25 g, 0.63 mmol) was added methyl iodide (0.200 mL, 3.23 mmol) and O(9)-allyl-N-(9-anthracenylmethyl)-cinchonidinium bromide (40 mg, 0.066 mmol). The solution was cooled to -78 °C and neat BTPP (0.289 mL, 0.95 mmol) was added. The resulting orange solution was stirred at -78 °C for 2 hours and allowed to reach -50 °C. After 2 hours at -50 °C, the solution was diluted with CH_2Cl_2 (10 mL), washed with water (10 mL), dried ($MgSO_4$), filtered, and evaporated to give the crude desired racemic alkylated imine as a yellow oil.

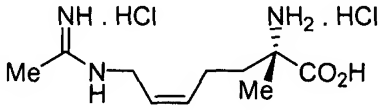
The crude material was dissolved in ethyl acetate (10 mL) and 1N HCl (10 mL) was added. The mixture was stirred for 1 hour at room temperature, and the organic layer was separated. The aqueous layer was neutralized with solid $NaHCO_3$ and extracted with ethyl acetate (2 x 30 mL). The organic layer was dried ($MgSO_4$), filtered and evaporated to give 0.16 g of the desired racemic 2-methylamino ester product as a yellow oil. The product was used in the next step without further purification. LCMS: *m/z* = 288.2 $[M+H]^+$.



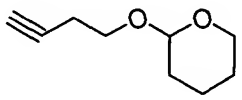
Example-KK-4) The racemic product from **Example-KK-3** is dissolved in water and acetic acid. Zinc dust is added, and the mixture is heated at 60 °C until HPLC analysis shows that little of the starting material remains. The Zn dust is filtered through celite from the reaction mixture, and the filtrate is concentrated. The crude material is purified by reverse-phase HPLC column chromatography. Fractions containing product are combined and concentrated affording the desired acetamidine product.

Example-KK) A solution of racemic **Example-KK-4** in 2.0 N HCl is refluxed for 1 h. The solvent is removed *in vacuo*. The resulting solid is dissolved in water and concentrated repeatedly from 1.0 N HCl to give the desired title (2*R/S*,5*E*)-2-amino-2-methyl-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride product.

Example LL

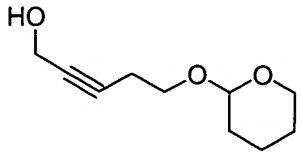


(2*S*,5*Z*)-2-amino-2-methyl-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride



4-[(Tetrahydropyranyl)oxy]butyne

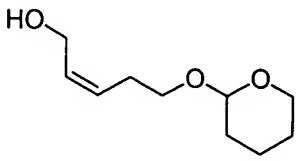
Example LL-1) A mixture of 4-dihydro-2H-pyridine (293.2 g 3.5 mol) and concentrated HCl (1.1 mL) was cooled to 5 °C. While continuing to cool externally, 3-butyn-1-ol (231.5 g, 3.3 mol) was added over a period of 30 minutes allowing the temperature to reach 50 °C. Reaction was held with mixing at room temperature for 2.5 hours before it was diluted with MTBE (1.0 L). The resulting mixture was washed with saturated sodium bicarbonate (2x150 mL). The organic phase was dried over sodium sulfate and concentrated under reduced pressure to afford 500 g (98% crude yield) of product; GC area% of 96%.



5-(Tetrahydro-pyran-2-yloxy)-pent-2-yn-1-ol

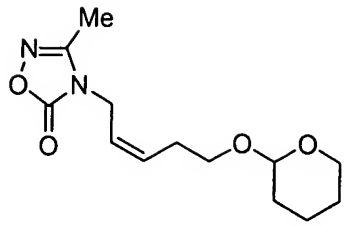
Example LL-2) To a solution of the 4-[(tetrahydropyranyl)oxy]butyne product of **Example LL-1** (50.0 g, 0.33 mol) in THF (125 mL) was added a solution of 2N EtMgCl in THF (242 mL, 0.48 mol) under a nitrogen atmosphere over a 30 minute period, allowing the temperature to rise to 48 °C. Mixture was further heated to 66 °C and was held at this temperature for 2 hours before cooling to ambient temperature. Paraformaldehyde (14.5 g, 0.48 mol) was added (small exotherm was observed) and the resulting mixture was heated to 45 °C. After 1 hour of controlling the temperature between 45-55 °C, the mixture turned clear. At this point, the mixture was heated up to 66 °C and stirred for 2.5 hours. Mixture was cooled to room temperature and saturated ammonium

chloride (125 mL) was added slowly over 30 minutes (strong exotherm was observed) keeping the temperature below 40 °C. The liquid phase was separated by decantation; ethyl acetate (250 mL) and brine (50 mL) were added. The organic phase was separated and washed with brine (2x50 mL) and water (1x50 mL). The organic layer was dried over sodium sulfate and concentrated under reduced pressure to afford 51 g of a lightly yellow colored oil (85% crude yield); GC area% = 88% title product, 6% starting material.



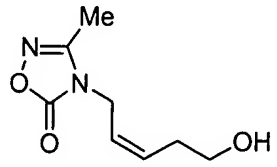
5-(Tetrahydro-pyran-2-yloxy)-pent-2-en-1-ol

Example LL-3) To a 500 mL Parr bottle, under a nitrogen atmosphere, was charged the 5-(tetrahydro-pyran-2-yloxy)-pent-2-yn-1-ol product of **Example LL-2** (40.2 g, 0.22 mol), Lindlar catalyst (2.0 g), ethanol (120 mL), hexane (120 mL), and 2,6-lutidine (457 mg). Reaction mixture was purged five times each with nitrogen and hydrogen gas. Parr bottle was pressurized with hydrogen to 5 psi and shaken until 98% of the theoretical hydrogen was consumed. Hydrogen was released from the vessel and the reaction was purged with nitrogen five times. Mixture was filtered through a pad of Solka Floc and the catalyst was rinsed with ethanol (2x50 mL). The filtrate and rinses were combined and concentrated under reduced pressure to afford 40.3 g (99% yield) of the title material as a yellow colored oil (GC area % = 96%).



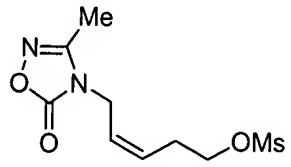
3-Methyl-4-[5-(tetrahydro-pyran-2-yloxy)-pent-2-enyl]-4H-[1,2,4]oxadiazol-5-one

Example LL-4) To a solution of the 5-(tetrahydro-pyran-2-yloxy)-pent-2-en-1-ol product of **Example LL-3** (11.8 g, 0.063 mol) in toluene (42 mL) was added) triethylamine (6.4 g, 0.063 mol). The mixture was cooled to –5 °C and methanesulfonyl chloride (7.3 g, 0.63 mol) was added via syringe at such rate as to keep the pot temperature below 10 °C. The mixture was allowed to warm to room temperature and stirred for two hours. The mixture was filtered by suction and rinsed on the filter with toluene (2x20 mL). The filtrate and washes were added to a mixture of the sodium salt of 3-methyl-1,2,4-oxadiazolin-5-one (8.6 g, 0.063 mol) in DMF (10 mL). The mixture was stirred with a mechanical stirrer and heated at 45 °C for 5 hours. Water (40 mL) was added and the mixture was stirred for 5 minutes and then the layers were separated. The toluene layer was washed with water (3x20 mL), dried over $MgSO_4$, and concentrated to afford 16.5 g (97.3%) of an orange colored crude product (area% GC consisted of 71% title product, 18% toluene, and 4% of an impurity).



4-(5-Hydroxy-pent-2-enyl)-3-methyl-4H-[1,2,4]oxadiazol-5-one

Example LL-5) To a solution the 3-methyl-4-[5-(tetrahydro-pyran-2-yloxy)-pent-2-enyl]-4H-[1,2,4]oxadi-az-ol-5-one product of **Example LL-4** (16 g, 0.06 mol) in methanol (48 mL) was added *p*-toluenesulfonic acid (0.34 g, 2.0 mmol). The mixture was stirred at room temperature for four hours. Sodium bicarbonate (0.27 g, 3.0 mmol) was added and the mixture was concentrated on a rotary evaporator. The residue was diluted with saturated $NaHCO_3$ (20 mL) and the resulting mixture was extracted with ethyl acetate (2x60 mL). Extracts were combined and washed with water (2x25 mL), dried over $MgSO_4$, and concentrated to afford 8.4 g of the crude, orange colored oil title product (area% GC= 80%).



Methanesulfonic acid 5-(3-methyl-5-oxo-[1,2,4]oxadiazol-4-yl)-pent-3-enyl ester

Example LL-6) To a solution of the 4-(5-Hydroxy-pent-2-enyl)-3-methyl-4H-[1,2,4]oxadiazol-5-one product of **Example LL-5** (8.27 g, 0.045 mol) in methylene chloride (33 mL) was added triethylamine (5.0 g, 0.49 mol). The mixture was cooled to –5 °C and methanesulfonyl chloride (5.5 g, 0.048 mol) was added at such rate as to keep the temperature below 8 °C. The cooling bath was removed and the mixture was stirred for 3 hours as it warmed up to room temperature. Water (15 mL) was added and the mixture was stirred for 5 minutes and then the layers were separated. The organic phase was washed

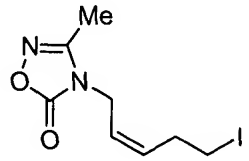
with water (10 mL), dried over $MgSO_4$, and concentrated to give a light amber colored residue. The residue was dissolved in ethyl acetate (8 mL) and kept at 5 °C overnight. Precipitated solids were filtered off by suction and rinsed on the filter with minimum volume of ethyl acetate and then air-dried on the filter to afford 6.8 g (58% yield) of the title product.

^{1}H NMR ($CDCl_3$) δ 5.76 (dtt, J=10.9, 7.5, 1.5 Hz, 1H), δ 5.59 (dtt, J=10.9, 7.0, 1.5 Hz, 1H), δ 4.31 (t, J=6.3 Hz, 2H), δ 4.27 (dd, J=7.0, 1.5 Hz, 2H), δ 3.04 (s, 3H), δ 2.67 (q, J=6.7 Hz, 2H), δ 2.28 (s, 3H)

^{13}C ($CDCl_3$) δ 159.0, 156.3, 129.9, 125.1, 68.4, 38.9, 37.2, 27.5, 10.2.

IR (cm^{-1}) 1758, 1605, 1342,1320,1170.

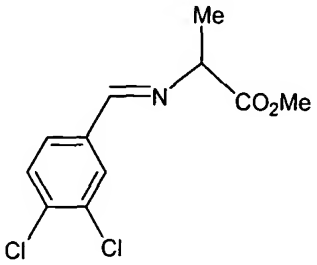
Anal. Calcd. for $C_9H_{14}N_2O_5S$: C, 41.21; H, 5.38; N, 10.68. Found: C, 41.15; H, 5.41; N, 10.51.



4-(5-Iodo-pent-2-enyl)-3-methyl-4H-[1,2,4]oxadiazol-5-one

Example LL-7) To a solution of the methanesulfonic acid 5-(3-methyl-5-oxo-[1,2,4]oxadiazol-4-yl)-pent-3-enyl ester product of **Example LL-6** (20.0 g, 0.076 mol) in acetone (160 ml) was added sodium iodide (17.15 g, 0.114 mol). The mixture was heated to reflux and was stirred for 3 hours. External heating was stopped and the mixture was held at room temperature overnight. Solids were removed by filtration and rinsed on the filter. The filtrate and washes were combined and concentrated and the heterogeneous residue was extracted with ethyl acetate (120 mL). The organic layer was washed with

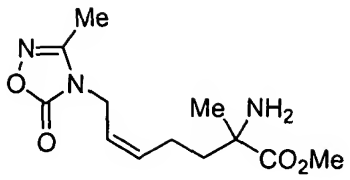
water (60 mL), 15% aqueous solution of sodium thiosulfate (60 mL) and water (60 mL); dried over $MgSO_4$ and concentrated under reduced pressure to afford 22.1 g (98% yield) of the title oil product.



2-[(3,4-Dichloro-benzylidene)-amino]-propionic acid methyl ester

Example LL-8) To a mechanically stirred slurry of L-alanine methyl ester hydrochloride (200.0 g, 1.43 mol) in methylene chloride (2.1 L) under a nitrogen atmosphere was added triethylamine (199.7 mL, 1.43 mol) over 12 min (during the addition solids partially dissolved and then reprecipitated). After 10 min, 3,4-dichlorobenzaldehyde (227.5 g, 1.30 mol) and magnesium sulfate (173.0 g, 1.43 mol) were added (temperature increased 6 °C over 30 min). After 2.5 h, the mixture was filtered. The filtrate was washed with water (1 x 1 L) and brine (1 x 500 mL), dried over sodium sulfate, filtered and concentrated to give 313.3 g, 92.4% yield of oil product.

^{1}H NMR (400 MHz, CDCl3) □8.25 (s, 1H), 7.91 (d, 1H), 7.58 (dd, 1H), 7.49 (d, 1H), 4.17 (t, 1H), 3.76 (s, 3H), 1.53 (d, 3H). Anal. Calcd for $C_{11}H_{11}Cl_2NO_2$: C, 50.79; H, 4.26; Cl, 27.26; N, 5.38. Found: C, 50.37; H, 4.10; Cl, 26.87; N, 5.38.



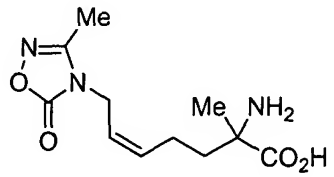
***Rac*-2-Amino-2-methyl-7-(3-methyl-5-oxo-[1,2,4]oxadiazol-4-yl)-hept-5-enoic acid methyl ester**

Example LL-9) Method 1. A solution of the product of **Example LL-7** (114.2 g, 0.39 mol) and the product of **Example LL-8** (151.5 g, 0.58 mol) in dimethylformamide (1.4 L) under nitrogen atmosphere was cooled to –8 °C. Lithium iodide (78.1 g, 0.58 mol) was then added in 3 equal portions over 19 min. The mixture was stirred for 20 min at –7 °C and then (tert-butylimino)-tris(pyr-rolidino)phosphorane (194.0 mL, 0.62) was added over 36 min (maximum temperature = -2.6 °C). After 10 min, the cooling bath was removed and the solution was stirred at ambient temperature for 1h. The mixture was then poured into cold water (1.4 L) and extracted with ethyl acetate (2 x 1.0 L). The combined organic layers were washed with water (2 x 400 mL) and brine. The ethyl acetate layer was treated with 1 N HCl (780 mL) and stirred for 1 h. The aqueous layer was separated and extracted with ethyl acetate (2 x 400 mL) and then neutralized with sodium bicarbonate (110 g). The mixture was extracted with ethyl acetate (1 x 500 mL). The organic layer was dried over sodium sulfate, filtered, concentrated and then treated with methyl t-butyl ether to give a crystalline product: first crop 14.4 g; second crop 6.6g (GC purity = 96.2 and 91.9%, respectively). The aqueous phase was saturated with sodium chloride and extracted with ethyl acetate (4 x 500 mL). The combined organic layers were dried over sodium sulfate, filtered, concentrated and then treated with methyl t-butyl ether to give a crystalline product: first crop 33.4 g; second crop 10.8 g (GC purity = 89.6 and 88.8%, respectively. Total crude yield 65.2 g, 62.4%.

Method 2. To a solution of the product of **Example LL-7** (20.7 g, 0.070 mol) and the product of **Example LL-8** (22.9 g, 0.088 mol) in dimethylformamide (207 mL) under a nitrogen atmosphere was added cesium carbonate (29.8 g, 0.092). The mixture was stirred at rt for 16 h and then diluted with water (300 mL) and extracted with ethyl acetate (2 x 200 mL). The combined ethyl acetate layers were washed with water (3 x 100 mL) and brine and then treated with 1 N HCl (184 mL). After 1 h, the layers were separated and the aqueous layer was extracted with ethyl acetate (3 x 100 mL) and then neutralized with sodium bicarbonate (15.5 g). The mixture was extracted with ethyl acetate (1 x 150 mL). The aqueous layer was saturated with sodium chloride and extracted with ethyl acetate (3 x 100 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated to give a yellow solid, 11.9 g, 62.9%; GC purity = 96.6%. The crude product was recrystallized from warm methyl t-butyl ether or ethyl acetate.

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.68 (m, 1H), 5.36 (m, 1H), 4.23 (d, 2H), 3.73 (s, 3H), 2.43 (s, 3H), 2.18 (m, 2H), 1.81 (m, 1H), 1.69 (s, br, 2H), 1.66 (m, 1H), (1.36, 3H)

^{13}C NMR (400 MHz, $CDCl_3$) δ 177.60, 159.01, 156.10, 135.12, 121.82, 57.48, 52.29, 40.12, 39.00, 26.62, 22.56, 10.41

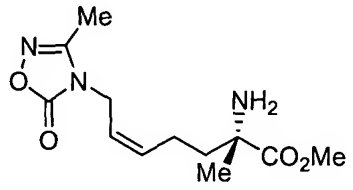


***Rac*-2-Amino-2-methyl-7-(3-methyl-5-oxo-[1,2,4]oxadiazol-4-yl)-hept-5-enoic acid**

Example LL-10) The product of **Example LL-9** (0.269g, 1 mmol) was dissolved in 5mL 2 N HCl and heated to reflux under argon. After refluxing for 6 hrs followed by stirring at room temperature for 72 hours, an aliquot was removed and checked by ^{1}H NMR. Approximately 6% of unreacted starting ester remained along with the desired product (verified by LC-MS). The aqueous portion was removed *in vacuo*, leaving 0.38g of a thick, amber oil. After purification via reverse phase chromatography, followed by lyophilization, one obtained 0.23g, 90.2% of the title compound as white, non-deliquescent solids.

Anal. Calcd. for $C_{11}H_{17}N_3O_4.0.77H_2O$: C, 49.09; H, 6.94; N, 15.61. Found: C, 48.71; H, 6.94; N, 15.98

Mass spec: M+1 = 256.



(2S,5Z)-2-Amino-2-methyl-7-(3-methyl-5-oxo-[1,2,4]oxadiazol-4-yl)-hept-5-enoic acid methyl ester

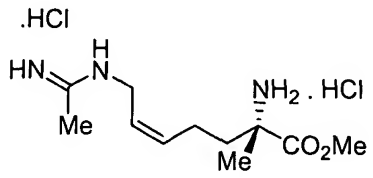
Example LL-11) The title compound (827.3g) was separated from its R enantiomer by preparative chiral chromatography using Novaprep 200 instrument with steady state recycling option. The material was dissolved in absolute ethanol at a concentration of 40 mg/ml and loaded on a 50x500 mm prepacked Chiral Technologies stainless steel column. The adsorbent was 20μ ChiralPak AD. The mobile phase was ethanol/triethylamine 100/0.1; the flow rate equaled 125 ml per min. The crude solution (25 mL) was loaded on

the column every 12 mins. A steady state recycling technique was used. Solvent was removed using a rotovap. The final product was isolated as gold oil which solidified on standing; 399.0 g (96.4% recovery).

^{1}H (400 MHz, CD_3OD) δ 5.68 (dtt, 1H, $J_{olefinic}$=10.7 Hz), 5.43 (dtt, 1H, $J_{olefinic}$=10.7 Hz), 4.82 (s, br, 2H), 4.28 (d, 2H, *J*=5.5 Hz), 3.73 (s, 3H), 2.27 (s, 3H), 2.26 (m, 1H), 2.14 (m,1H), 1.82 (ddd, 1H, *J*=13.6,11.3, 5.4 Hz), 1.67 (ddd, 1H, *J*=13.6, 11.2, 5.5 Hz), 1.34 (s, 3H)

^{13}C NMR (400 MHz, CD_3OD) δ 178.49, 161.13, 158.70, 135.92, 123.47, 58.55, 52.77, 41.38, 39.96, 26.23, 23.47, 10.23

Anal. Calcd for $C_{12}H_{19}N_3O_4$: C, 53.52; H, 7.11; N, 15.60. Found: C 52.35; H, 7.20; N, 15.60.



(2S,5Z)-7-Acetimidoylamino-2-amino-2-methyl-hept-5-enoic acid methyl ester, dihydrochloride hydrate

Example LL-12) To a solution of the product of **Example LL-11** (114.5 g, 0.425 mol) in methanol (2.4 L) was added the solid dibenzoyl-L-tartaric acid (152.5 g, 0.425 mol) and 88% formic acid (147 mL, 3.428 mol) at ambient temperature. A slurry of Lindlar catalyst, 5 wt% palladium on calcium carbonate poisoned with lead acetate (37.9 g), in methanol (200 mL) was prepared under nitrogen. The solution of starting material was then added at ambient temperature to the light grey catalyst slurry followed by a methanol rinse (200 mL). The heterogeneous reaction mixture was heated at 45 °C for 1

½ hours. Steady gas evolution was observed starting at about 40 °C, which indicated the ongoing reaction. The mixture was cooled in an ice/water bath and then filtered through a plug of Supercell HyFlo. The yellow solution was concentrated *in vacuo* to give a viscous oil, which was dissolved and partitioned between 2 N aqueous HCl (2 L) and ethyl acetate (0.8 L). Layers were separated and the aqueous layer was washed once with ethyl acetate (0.8 L). Solvent and volatiles were removed in vacuo at elevated temperatures (= 70 °C). The intermediate product was used in next the step without further purification or characterization. LC-MS $[M+H]^+$ = 228.

Example LL) The crude product of **Example LL-12** (170 g) was dissolved in 2 N aqueous HCl (1 L). The resulting orange solution was refluxed overnight before it was allowed to cool back to ambient temperature. The reaction mixture was concentrated to about 1/3 of its volume, and the acidic solution was passed through a solid phase extraction cartridge (25 g of C18 silica) to remove color and other impurities. Solvent was removed *in vacuo* (= 70 °C) to give 208 g of crude product as yellowish gum.

The crude gum (31.3 g) was taken up in water (250 mL) and the material was loaded onto a pretreated ion exchange column packed with the acidic resin Dowex 50WX4-400 (about 600 g). The resin was first washed with water (1 L), then with dilute aqueous HCl (1 L of 10/90 v/v conc. HCl/water). The product was eluted off the resin with higher ion strength aqueous HCl (1.5 L of 20/90 v/v to 25/75 v/v conc. HCl/water). The aqueous solvent was removed *in vacuo* (= 70 °C), and the gummy residue was taken up in 4 vol% aqueous trifluoroacetic acid (100 mL). The aqueous solvent was removed *in vacuo* (= 70 °C), and the procedure was repeated once more. The residue was then dried under high vacuum to give 32.2 g of gum as the trifluoroacetic acid salt.

Crude (2S,5Z)-7-acetimidoylamino-2-amino-2-methyl-hept-5-enoic acid, ditrifluoroace-tic acid salt hydrate (32.2 g) was purified by reverse-phase

preparative chromatography. The crude was dissolved in 0.1% aqueous TFA (50 ml) and loaded onto a 2-inch ID x 1 meter stainless steel column packed with adsorbent (BHK polar W/S, 50 □, 1.16 kg). The product was eluted at a flow rate of 120 mL/min with a step gradient from 0.1% aqueous TFA to 25/75/0.1 acetonitrile/water/TFA. The loading ratio was 36:1 w/w silica to sample. Solvent was removed *in vacuo*, and the material was converted into the HCl salt by repeated rinses with dilute aqueous HCl and solvent removals *in vacuo*. Drying under high vacuum gave 27.4 g of the title dihydrochloride hydrate as yellowish gum.

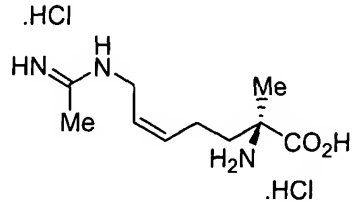
LC-MS $[M+H]^+$ = 214.16 Da

^{1}H NMR (D_2O, δ: 1.48 (s, 3H), 1.8-1.9 (AB, 2H), 2.10 (s, 3H), 2.01/2.12 (AB, 2H), 3.78 (d, 2H), rotamere 3.87 (d, 2H), 5.6/5.5 (dt, 2H, 11 Hz)

^{13}C NMR (D_2O) δ: 18.7, 21.5, 21.6, 36.4, 39.1, 59.8, 122.6, 134.3, 164.5, 173.7

Elemental Anal. Calcd. for $C_{10}H_{19}N_3O_2 \cdot 2.2HCl \cdot 2\ H_2O$: C, 36.21; H, 8.33; N, 12.67; Cl 23.51. Found: C, 36.03; H, 7.72; N, 12.67; Cl, 23.60.

Example MM



(2*R*,5*Z*)-2-amino-2-methyl-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride

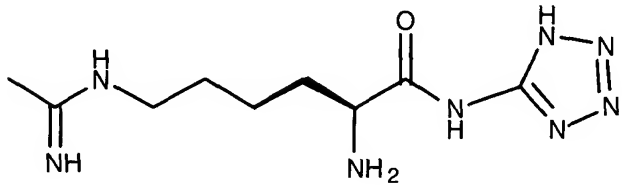
The R-enantiomer isolated during the separation described in **Example LL-11** (1.13g, 4.2 mmol) was dissolved in 11 mL 25% aqueous acetic acid and heated to 60 °C. Zinc dust (1.10g) was then added in 4 equal portions at 30-minute intervals. After heating for a total of 3 hours, an aliquot was removed and checked by LC-MS, which indicated only a trace of unreacted starting material remaining, along with desired product. The mixture was cooled to room temperature, filtered and stripped *in vacuo*, leaving 2.31 g of a slushy white solid. The methyl ester was hydrolysed with dilute hot HCl to the title compound. After purification by reverse phase chromatography followed by lyophilization, 0.31g of the title compound as a glassy solid was obtained.

Anal. Calcd. for $C_{10}H_{19}N_3O_2.1.22$ HCl.1.15 H_2O: C, 46.13; H, 8.15; N, 15.09; Cl, 15.53.

Found: C, 46.38; H, 8.51; N, 15.13; Cl, 15.80

Mass spec: M+1 = 214

Example NN



2S-amino-6-[(1-iminoethyl)amino]-N-(1H-tetrazol-5-yl) hexanamide, hydrate, dihydrochloride

NN-1 To a stirring solution of Boc-L-Lys(Cbz)-OH (5 g, 13.18 mmol), 5-aminotetrazole monohydrate (1.36 g, 13.18 mmol) and N,N-diisopropylethylamine (DIPEA) (5.1 g, 6.9 mL, 39.54 mmol) in 20 mL of dimethylformamide (DMF) at ambient temperature was added benzotriazol-1-yl-oxy-tris-(dimethylamino)phosphonium hexafluorophosphate (BOP) (6.4 g, 14.49 mmol).

After being stirred for 1 h, the reaction mixture was concentrated under vacuum. The residue was distributed between 60 mL of ethyl acetate (EtOAc) and 50 mL of water. The layers were separated. The organic layer was washed with 50 mL of 1M $KHSO_4$ solution and 2 times with 50 mL of water. The product started to precipitate and the suspension was concentrated in vacuum giving 9 g of crude compound. After drying, the product was purified by boiling in methylene chloride followed by filtration, giving 3.7 g of 1A (62.7%). The compound was characterized by ^1H NMR.

NN-2 (2 g, 4.5 mmol) was reduced under catalytic hydrogenation conditions using Pd black at 5 psi in 50% EtOH/AcOH solution for 12 h, giving 1.55 g (100%) of **NN-2**. The compound was characterized by ^1H NMR.

NN-3 To a stirring solution of **NN-2** (1.55 g, 4.15 mmol) and methyl acetimidate hydrochloride (0.91g, 8.31 mmol) in 25 mL of DMF was added triethylamine (TEA) (1.26 g, 1.74 mL, 12.45 mmol). After being stirred 16 h at ambient temperature, the reaction mixture was filtered from triethylamine hydrochloride and the filtrate was concentrated in vacuum. The residue was dissolved in 50% AcOH and lyophilized. The crude product (2 g) was purified using reverse-phase chromatography on a C-18 column giving 0.9 g (52.3%) of 1C. The product was characterized by ^1H NMR.

NN-4 (0.9 g, 2.17 mmol) was dissolved in 30 mL of acetic acid and 3 mL of 4 N HCl/dioxane were added. The reaction was stirred for 20 min. at ambient temperature then 150 mL of ethyl ether were added. After 2 h, the precipitate was filtered, washed with ethyl ether, and dried giving 0.78 g of **1** (96%). Anal. Calcd. for $C_9H_{18}N_8O$,2HCl, 1.25H_2O: C,30.91; H, 6.48; N, 32.04; Cl, 20.27. Found: C, 31.64; H, 6.43; N, 32.19; Cl, 20.19. DSC mp 144.9° C.

Example **NN** is a more potent i-NOS inhibitor than 2S-amino-6-[(1-iminoethyl)amino]hexanamide (NIL amide) or NIL dimethylamide. Example **1** is also more selective. Example **NN** is a nicely crystalline product as are all its intermediates. In contrast, NIL is a glass, which makes it difficult to handle.

c. Biological Data

Some or all of the following assays are used to demonstrate the nitric oxide synthase inhibitory activity of the invention's compounds as well as demonstrate the useful pharmacological properties.

Citrulline Assay for Nitric Oxide Synthase

Nitric oxide synthase (NOS) activity can be measured by monitoring the conversion of L-[2,3-^{3}H]-arginine to L-[2,3-^{3}H]-citrulline (Bredt and Snyder, Proc. Natl. Acad. Sci. U.S.A., 87, 682-685, 1990 and Moore et al, J. Med. Chem., 39, 669-672, 1996). Human inducible NOS (hiNOS), human endothelial constitutive NOS (hecNOS) and human neuronal constitutive NOS (hncNOS) are each cloned from RNA extracted from human tissue. The cDNA for human inducible NOS (hiNOS) is isolated from a λcDNA library made from RNA extracted from a colon sample from a patient with ulcerative colitis. The cDNA for human endothelial constitutive NOS (hecNOS) is isolated from a λcDNA library made from RNA extracted from human umbilical vein endothelial cells (HUVEC) and the cDNA for human neuronal constitutive NOS (hncNOS) is isolated from a λcDNA library made from RNA extracted from human cerebellum obtained from a cadaver. The recombinant enzymes are expressed in Sf9 insect cells using a baculovirus vector (Rodi et al, in The Biology of Nitric Oxide, Pt. 4: Enzymology, Biochemistry and Immunology; Moncada, S., Feelisch, M., Busse, R., Higgs, E., Eds.; Portland Press Ltd.: London, 1995; pp 447-450). Enzyme activity is isolated from soluble cell extracts and partially purified by DEAE-Sepharose chromatography. To measure NOS activity, 10 µL of enzyme is added to 40 µL of 50 mM Tris (pH 7.6) in the presence or absence of test compounds and the reaction initiated

by the addition of 50 µL of a reaction mixture containing 50mM Tris (pH 7.6), 2.0 mg/mL bovine serum albumin, 2.0 mM DTT, 4.0 mM $CaCl_2$, 20 µM FAD, 100 µM tetrahydrobiopterin, 0.4 mM NADPH and 60 µM L-arginine containing 0.9 µCi of L-[2,3-^{3}H]-arginine. The final concentration of L-arginine in the assay is 30 µM. For hecNOS or hncNOS, calmodulin is included at a final concentration of 40-100 nM. Following incubation at 37°C for 15 minutes, the reaction is terminated by addition of 400 µL of a suspension (1 part resin, 3 parts buffer) of Dowex 50W X-8 cation exchange resin in a stop buffer containing 10 mM EGTA, 100 mM HEPES, pH 5.5 and 1 mM L-citrulline. After mixing the resin is allowed to settle and L-[2,3-^{3}H]-Citrulline formation is determined by counting aliquots of the supernatant with a liquid scintillation counter. Results are reported in **Table I** as the IC_{50} values of compounds for hiNOS, hecNOS and hncNOS.

Raw Cell Nitrite Assay

RAW 264.7 cells can be plated to confluency on a 96-well tissue culture plate grown overnight (17h) in the presence of LPS to induce NOS. A row of 3-6 wells can be left untreated and served as controls for subtraction of nonspecific background. The media can be removed from each well and the cells washed twice with Kreb-Ringers-Hepes (25 mM, pH 7.4) with 2 mg/ml glucose. The cells are then placed on ice and incubated with 50 µL of buffer containing L-arginine (30 µM) +/- inhibitors for 1h. The assay can be initiated by warming the plate to 37° C in a water bath for 1h. Production of nitrite by intracellular iNOS will be linear with time. To terminate the cellular assay, the plate of cells can be placed on ice and the nitrite-containing buffer removed

and analyzed for nitrite using a previously published fluorescent determination for nitrite. (T. P. Misko et al, *Analytical Biochemistry,* 214, 11-16 (1993).

Human cartilage explant assay

Bone pieces are rinsed twice with Dulbecco's Phosphate Buffered Saline (GibcoBRL) and once with Dulbecco's Modified Eagles Medium (GibcoBRL) and placed into a petri dish with phenol red free Minimum Essential Medium (MEM) (GibcoBRL). Cartilage was cut into small explants of approximately 15-45 mg in weight and one or two explants per well are placed into either 96 or 48 well culture plates with 200-500 µL of culture media per well. The culture media was either a custom modification of Minimum Essential Medium(Eagle) with Earle's salts (GibcoBRL) prepared without L-Arginine, without L-Glutamine and without phenol red or a custom modification of serumless Neuman and Tytell (GibcoBRL) medium prepared without L-arginine, without insulin, without ascorbic acid, without L-glutamine and without phenol red. Both are supplemented before use with 100 µM L-Arginine (Sigma), 2 mM L-glutamine, 1X HL-1 supplement (BioWhittaker), 50 mg/ml ascorbic acid (Sigma) and 150 pg/ml recombinant human IL-1β (RD Systems) to induce nitric oxide synthase. Compounds are then added in 10 µL aliquots and the explants incubated at 37° C with 5% CO_2 for 18-24 hours. The day old supernatant is then discarded and replaced with fresh culture media containing recombinant human IL-1β and compound and incubated for another

20-24 hours. This supernatant is analyzed for nitrite with a fluorometric assay (Misko et al, *Anal. Biochem.*, 214, 11-16, 1993). All samples are done in quadruplicate. Unstimulated controls are cultured in media in the absence of recombinant human IL-1β. IC_{50} values (**Table I**) are determined from plotting the percent inhibition of nitrite production at six different concentrations of inhibitor.

Table I shows examples of biological activity for some of the compounds of the present invention.

TABLE I

Biological Activity: Values represent averages across all experiments and all lots studied.

Example Number of Compound	**hiNOS IC_{50} (μM)**	**hecNOS IC_{50} (μM)**	**hncNOS IC_{50} (μM)**	**Human Cartilage IC_{50} (μM)**
Example A	0.36	68	3.6	0.1
Example B	2.2	195	21	0.2
Example C	12	303	105	
Example D	8.6	112	65	2.5
Example E	<5	279	29	
Example I	3.1	77	15	0.7
Example J	4.4	302	58	8.2

Example K	74	266	86	
Example L	197	1100	539	
Example M	3.4	78	17	
Example N	0.9	26	6.0	
Example O	7.2	>100	36	0.7
Example P	12	>100	181	
Example Q	12	1080	220	
Example S	172	1490	523	
Example T	0.9	89	8	0.1
Example U	20	418	150	
Example V	<3	>30	>3	<10
Example W	<5	>150	>10	>30
Example X	<3	>15	>3	<10
Example Y	<3	>30	>3	<10
Example Z	<3	>15	>3	<10
Example AA	<3	>5	<3	<3
Example BB	<10	>25	<10	
Example CC	2.9	29	9.9	0.5
Example DD	10	74	31	1.8
Example EE	1.4	18	5.8	0.5

Example FF	16	86	45	
Example GG	34	386	122	
Example HH	0.4	37	7.6	0.4
Example JJ	56	352	584	
Example KK	0.57	52	13	
Example LL	0.7	31	12	0.8
Example MM	121	1930	1480	
Example NN	21.4	2425		

In Vivo Assay

Rats can be treated with an intraperitoneal injection of 1-12.5 mg/kg of endotoxin (LPS) with or without oral administration of the nitric oxide synthase inhibitors. Plasma nitrite/nitrate levels can be determined 5 hours post-treatment. The results can be used to show that the administration of the nitric oxide synthase inhibitors decreases the rise in plasma nitrite/nitrate levels, a reliable indicator of the production of nitric oxide induced by endotoxin. As shown in **Table II**, Example A ((2*S*,5*E*)-2-amino-6-fluoro-7-[(1-iminoethyl)amino]-5-heptenoic acid, dihydrochloride) inhibited the LPS-induced increase in plasma nitrite/nitrate levels with an observed ED_{50} value of <0.1 mg/kg,demonstrating the ability to inhibit inducible nitric oxide synthase activity *in vivo*.

TABLE II

ED_{50}'s for Compounds Determined in Endotoxin-Treated Rats

All compounds administered orally unless otherwise noted.

Compound	ED_{50} (mg/kg)
Example A	< 0.1
Example D	>10
Example G	< 0.1
Example H	< 0.3
Example V	<3
Example W	>10
Example X	<5
Example Y	<3
Example Z	<5
Example AA	<10
Example CC	<3
Example EE	0.2
Example HH	0.4
Example KK	0.3
Example LL	0.3

Assay for Time Dependent Inhibition

Compounds are evaluated for time dependent inhibition of human NOS isoforms by preincubation of the compound with the enzyme at 37° C in the presence of the citrulline enzyme assay components, minus L-arginine, for times ranging from 0-60 minutes. Aliquots (10 µL) are removed at 0, 10, 21

and 60 minutes and immediately added to a citrulline assay enzyme reaction mixture containing L-[2,3-^{3}H]-arginine and a final L-arginine concentration of 30 μM in a final volume of 100 μL. The reaction is allowed to proceed for 15 minutes at 37° C and terminated by addition of stop buffer and chromatography with Dowex 50W X-8 cation exchange ion exchange resin as described for the citrulline NOS assay. The % inhibition of NOS activity by an inhibitor was taken as the percent inhibition in activity compared to control enzyme preincubated for the same time in the absence of inhibitor. Data shown in Table III is the % inhibition after 21 and 60 minutes preincubation of inhibitor with enzyme.

TABLE III

Example No.	hiNOS	hecNOS	hncNOS
V	75%@2.8μM@21min 76%@2.8μM@60min	11%@33μM@21min 11%@33μM@60min	0%@5μM@21min 0%@5μM@60min
W	34%@4.2μM@21min 38%@4.2μM@60min	9%@173μM@21min 0%@173μM@60min	0%@13μM@21min 0%@13μM@60min
X	86%@2.2μM@21min 85%@2.2μM@60min	18%@15μM@21min 16%@15μM@60min	0%@3μM@21min 0%@3μM@60min
Y	75%@2.8μM@21min 76%@2.8μM@60min	11%@33μM@21min 11%@33μM@60min	0%@5μM@21min 0%@5μM@60min
Z	86%@2.2μM@21min 85%@2.2μM@60min	18%@15μM@21min 16%@15μM@60min	0%@3μM@21min 0%@3μM@60min
AA	96%@2.2μM@21min 97%@2.2μM@60min	58%@5.7μM@21min 55%@2.2μM@60min	34%@0.9μM@21mi 0%@0.9μM@60min

Assay of Anti-Cytotoxic Effect of Selective iNOS Inhibitors on Human Gastric Epithelial Cells Infected with H. Pylori

To determine the anti-cytotoxic effects of selective iNOS inhibitors on

gastric epithelial cells, cells obtained from human gastric epithelial cell line AGS (gastric adenocarcinoma, ATCC CRL 1739; available from American Type Culture Collection) are grown in RPMI-1640 medium supplemented with 10% fetal bovine serum and antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin). Cells are seeded onto a 24 well culture plate at a density of 4 x 10^5 cells per well in a volume of of 1 ml and cultured overnight to reach 80% confluency. Before stimulation, cells are washed three times with 1 ml of fresh culture medium containing no antibiotics. Cells are then cultured in the presence of *H. pylori* at a bacterium to cell ratio of 300:1 for 12-36 hours, with (treated) or without (control) treatment with an iNOS selective inhibitor at a dose of, for example, 1 µM to 1 mM. As an index of cytotoxicity, cell number is assessed by trypan blue exclusion analysis. Viable cells are counted at a fixed time point, or multiple fixed time points, within the 12-36 hour period. Cell numbers in control and treated cell samples are compared.

Assays of Anti-Apoptotic Effect of Selective iNOS Inhibitors on Human Gastric Epithelial Cells Infected with H. Pylori

AGS cells are cultured as described immediately above. AGS cells (4 x 10^5/well) are plated onto glass coverslips in 24 well plates, and are treated with (treated) or without (control) an iNOS selective inhibitor and cultured in the presence of *H. pylori* (at a bacterium to cell ratio of 300:1) for 24 hours. Cells are washed twice with PBS, cell monolayers fixed with 4% paraformaldehyde and cells stained with a DNS-specific dye such as Hoechst 33258. As an index of apoptosis, DNA fragmentation is assessed using fluoresence microscopy. DNA fragmentation, and numbers of apoptotic cells in treated and control samples are determined and compared.

d. Dosages, Formulations and Routes of Administration

Many of the iNOS selective inhibitor compounds useful in the methods of the present invention can have at least two asymmetric carbon atoms, and therefore include racemates and stereoisomers, such as diastereomers and enantiomers, in both pure form and in admixture. Such stereoisomers can be prepared using conventional techniques, either by reacting enantiomeric starting materials, or by separating isomers of compounds of the present invention. Isomers may include geometric isomers, for example cis-isomers or trans-isomers across a double bond. All such isomers are contemplated among the compounds useful in the methods of the present invention. The methods also contemplate use of tautomers, salts, solvates and prodrugs of iNOS selective inhibitor compounds.

For the methods of the present invention, suitable routes of administration of the selective iNOS inhibitors include any means that produce contact of these compounds with their site of action in the subject's body, for example in the gastrointestinal tract, including the esophagus, stomach, and intestines of a mammal such as a human. More specifically, suitable routes of administration include oral, intravenous, subcutaneous, rectal, topical, buccal (i.e. sublingual), intramuscular, and intradermal. In an exemplary embodiment, the selective iNOS inhibitors are orally administered.

For the prophylaxis or treatment of conditions of the gastrointestinal tract, including inflammatory bowel disease including Crohn's disease and ulcerative colitis, peptic ulcer disease including gastric ulceration and duodenal ulceration, gastritis, colitis, ileitis, esophagitis, paralytic ileus, diarrhea and irritable bowel syndrome, the methods include use of an iNOS selective inhibitor as the compound per se, or as pharmaceutically acceptable salts thereof. The methods of the present invention also include use of an iNOS selective inhibitor in combination with an antimicrobial agent, in combination with an antisecretory agent, or in combination with both an antimicrobial agent

and an antisecretory agent. The term "pharmaceutically-acceptable salts" embraces salts commonly used to form alkali metal salts and to form addition salts of free acids or free bases. The nature of the salt is not critical, provided that it is pharmaceutically acceptable. Pharmaceutically acceptable salts are particularly useful as products of the methods of the present invention because of their greater aqueous solubility relative to a corresponding parent or neutral compound. Such salts must have a pharmaceutically acceptable anion or cation. Suitable pharmaceutically-acceptable acid addition salts of compounds of the present invention may be prepared from inorganic acid or from an organic acid. Examples of such inorganic acids are hydrochloric, hydrobromic, hydroiodic, nitric, carbonic, sulfuric and phosphoric acid. Appropriate organic acids include from aliphatic, cycloaliphatic, aromatic, araliphatic, heterocyclic, carboxylic and sulfonic classes of organic acids, examples of which are formic, acetic, propionic, succinic, glycolic, gluconic, lactic, malic, tartaric, citric, ascorbic, glucoronic, maleic, fumaric, pyruvic, aspartic, glutamic, benzoic, anthranilic, mesylic, salicylic, p-hydroxybenzoic, phenylacetic, mandelic, embonic (pamoic), methanesulfonic, ethylsulfonic, benzenesulfonic, sulfanilic, stearic, cyclohexylaminosulfonic, algenic, galacturonic acid. Suitable pharmaceutically-acceptable base addition salts of compounds of the present invention include metallic salts made from aluminum, calcium, lithium, magnesium, potassium, sodium and zinc or organic salts made from N,N'-dibenzylethyleneldiamine, choline, chloroprocaine, diethanolamine, ethylenediamine, meglumine (N-methylglucamine) and procain. Suitable pharmaceutically acceptable acid addition salts of the compounds of the present invention when possible include those derived from inorganic acids, such as hydrochloric, hydrobromic, hydrofluoric, boric, fluoroboric, phosphoric, metaphosphoric, nitric, carbonic (including carbonate and hydrogen carbonate anions), sulfonic, and sulfuric acids, and organic acids such as acetic, benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric, gluconic, glycolic,

isothionic, lactic, lactobionic, maleic, malic, methanesulfonic, trifluoromethanesulfonic, succinic, toluenesulfonic, tartaric, and trifluoroacetic acids. The chloride salt is particularly preferred for medical purposes. Suitable pharmaceutically acceptable base salts include ammonium salts, alkali metal salts such as sodium and potassium salts, and alkaline earth salts such as magnesium and calcium salts. All of these salts may be prepared by conventional means from the corresponding conjugate base or conjugate acid of the compounds of the present invention by reacting, respectively, the appropriate acid or base with the conjugate base or conjugate acid of the compound.

In one embodiment, the iNOS selective inhibitors useful in the methods of the present invention are presented with an acceptable carrier in the form of a pharmaceutical combination. The carrier must be acceptable in the sense of being compatible with the other ingredients of the pharmaceutical combination and must not be deleterious to the subject. Suitable forms for the carrier include solid or liquid or both, and in an exemplary embodiment the carrier is formulated with the therapeutic compound as a unit-dose combination, for example as a tablet that contains from about 0.05% to about 95% by weight of the active compound. In alternative embodiments, other pharmacologically active substances are also present, including other compounds of the present invention. The pharmaceutical compounds of the present invention are prepared by any of the well-known techniques of pharmacy, consisting essentially of admixing the ingredients.

Preferred unit dosage formulations are those containing an effective dose, as herein below described, or an appropriate fraction thereof, of one or more of the therapeutic compounds of the combinations.

In general, a total daily dose of an iNOS selective inhibitor is in the range of about 0.001 mg/kg body weight/day to about 2500 mg/kg body weight/day. The dose range for adult humans is generally from about 0.005 mg to about 10

g per day. Tablets or other forms of presentation provided in discrete units may conveniently contain an amount of a therapeutic compound that is effective at such dosage, or at a multiple of the same. For instance, selective iNOS inhibitory compounds used in the present invention can be presented in units containing 5 mg to 500 mg, and typically around 10 mg to about 200 mg.

In general, as an anti-microbial compound in combination with an iNOS selective inhibitor, a total daily dose of an antibiotic compound for adult humans is in the range of about 0.1 g per day to about 15 g per day. Typically a total daily dose for adult humans is the range of about 0.25 g per day to about 4 g per day.

In general, as an anti-microbial compound in combination with an iNOS selective inhibitor, a total daily dose of a bismuth compound for adult humans is in the range of about 100 mg per day to about 1000 mg/day, and typically about 500 mg/day.

In general, as an antisecretory compound in combination with an iNOS selective inhibitor, a total daily dose of an H_2 receptor anatagonist compound for adult humans is in the range of about 10 mg per day to about 1000 mg per day, and typically about 300 mg/ day to about 800 mg/day.

In general, as an antisecretory compound in combination with an iNOS selective inhibitor, a total daily dose of a proton pump inhibitor compound for adult humans is in the range of about 10 mg/ day to about 200 mg/ day. Typically a total daily dose is in the range of about 20 mg/day to about 60 mg/day of omeprazole, or about 15 mg/day to about 30 mg/day for lansoprazole.

Double or triple therapies using combinations of anti-microbial agents and antisecretory agents, in combination with an iNOS selective inhibitor, are also useful in the methods of the present invention. A double therapy includes, for example, a combination of an antisecretory agent such as omeprazole with an antibiotic such as clarithromycin or amoxicillin. Triple

therapy includes, for example, administration of metronidazole, a bismuth compound and either tetracycline or amoxicillin. Another triple therapy useful in the methods of the present invention is ranitidine plus a bismuth compound and an antibiotic compound.

In the case of pharmaceutically acceptable salts of the therapeutic compounds, the weights indicated above refer to the weight of the acid equivalent or the base equivalent of the therapeutic compound derived from the salt.

For the methods herein described, it should be understood that the amount of a selective iNOS inhibitory compound that is required to achieve the desired biological effect depends on a number of factors, including the specific individual compound or compounds chosen, the specific use, the route of administration, the clinical condition of the subject, and the age, weight, gender, and diet of the subject. Similarly, it should be understood that the total amount of a selective iNOS inhibitory compound in combination with any other thereapeutic agent or agents that is required to achieve the desired biological effect depends on a number of factors, including the specific individual compound or compounds chosen, the specific use, the route of administration, the clinical condition of the subject, and the age, weight, gender, and diet of the subject.

The daily doses described in the preceding paragraphs for the various therapeutic compounds are administered in a single dose, or in proportionate multiple subdoses. Subdoses are administered from two to six times per day. In one embodiment, doses are administered in sustained release form effective to obtain the desired biological effect.

Oral delivery according to the methods of the present invention can include formulations, as are well known in the art, to provide prolonged or sustained delivery of the drug to the gastrointestinal tract by any number of mechanisms. These include, but are not limited to, pH sensitive release from

the dosage form based on the changing pH of the small intestine, slow erosion of a tablet or capsule, retention in the stomach based on physical properties of the formulation, bioadhesion of the dosage form to the mucosal lining of the intestinal tract, or enzymatic release of the active drug from the dosage form.

Oral delivery according to the methods of the present invention can be achieved using a solid, semi-solid or liquid dosage form. Suitable semi-solid and liquid forms include, for example, a syrup or liquid contained in a gel capsule.

To practice the methods of the present invention, pharmaceutical compositions suitable for oral administration can be presented in discrete units, such as capsules, cachets, lozenges, or tablets, each containing a predetermined amount of at least one of the therapeutic compounds useful in the methods of the present invention; as a powder or in granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water or water-in-oil emulsion.

e. Examples of Embodiments

The following non-limiting examples serve to illustrate various pharmaceutical compositions suitable for practicing the treatment methods of the present invention.

EXAMPLE 1: Pharmaceutical Compositions

100 mg tablets of the composition set forth in Table IV can be prepared for oral administration using wet granulation techniques:

Table IV

Ingredient	Weight (mg)
Compound II	**25**
Lactose	54

Microcrystalline Cellulose	15
Hydroxypropyl Methylcellulose	3
Croscarmelose Sodium	2
Magnesium Stearate	1
Total Tablet Weight	**100**

EXAMPLE 2: Pharmaceutical Compositions

100 mg tablets of the composition set forth in Table V can be prepared using direct compression techniques:

Table V

Ingredient	**Weight (mg)**
Compound I	**25**
Microcrystalline Cellulose	69.5
Colloidal Silicon Dioxide	0.5
Talc	2.5
Croscarmelose Sodium	0.5
Magnesium Stearate	1
Total Tablet Weight	**100**

The methods of the present invention also contemplate combination therapy using selective iNOs inhibitors in combination with an anti-microbial agent or combination of anti-microbial agents, using selective iNOS inhibitors in combination with antisecretory agents, and using selective iNOS inhibitors in combination with both anti-microbial agents and antisecretory agents.

EXAMPLE 3: Pharmaceutical Compositions

100 mg tablets of the composition set forth in Table VI can be prepared

for oral administration using wet granulation techniques:

Table VI

Ingredient	Weight (mg)
Compound II	**5**
Omeprazole	20
Lactose	54
Microcrystalline Cellulose	15
Hydroxypropyl Methylcellulose	3
Croscarmelose Sodium	2
Magnesium Stearate	1
Total Tablet Weight	**100**

EXAMPLE 4: Pharmaceutical Compositions

100 mg tablets of the composition set forth in Table VII can be prepared using direct compression techniques:

Table VII

Ingredient	Weight (mg)
Compound II	**5**
Omeprazole	20
Microcrystalline Cellulose	69.5
Colloidal Silicon Dioxide	0.5
Talc	2.5
Croscarmelose Sodium	0.5
Magnesium Stearate	1
Total Tablet Weight	**100**

EXAMPLE 5: Pharmaceutical Compositions

150 mg tablets of the composition set forth in Table VIII can be prepared for oral administration using wet granulation techniques:

Table VIII

Ingredient	Weight (mg)
Compound II	**5**
Amoxicillin	50
Lactose	65
Microcrystalline Cellulose	20
Hydroxypropyl Methylcellulose	5
Croscarmelose Sodium	3
Magnesium Stearate	2
Total Tablet Weight	**150**

EXAMPLE 6: Pharmaceutical Compositions

150 mg tablets of the composition set forth in Table IX can be prepared using direct compression techniques:

Table IX

Ingredient	Weight (mg)
Compound II	**10**
Amoxicillin	50
Microcrystalline Cellulose	81
Colloidal Silicon Dioxide	1.0
Talc	5.0
Croscarmelose Sodium	1.0
Magnesium Stearate	2
Total Tablet Weight	**150**

The examples described herein can be performed by substituting the generically or specifically described therapeutic compounds or inert ingredients for those used in the preceding examples.

The explanations and illustrations presented herein are intended to acquaint others skilled in the art with the invention, its principles, and its practical application. Those skilled in the art may adapt and apply the invention in its numerous forms, as may be best suited to the requirements of a particular use. Accordingly, the specific embodiments of the present invention as set forth are not intended as being exhaustive or limiting of the invention.